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CONTENTS

EDITORIAL POLICY

Cereal Chemistry publishes scientific papers dealing with raw materials, processes, or products of the cereal industries, or with analytical procedures, technological tests, or fundamental research, related thereto. Papers must be based on original investigations, not previously described elsewhere, which make a definite contribution to existing knowledge.

Cereal Chemistry gives preference to suitable papers presented at the Annual Meeting of the American Association of Cereal Chemists, or submitted directly by members of the Association. When space permits, papers are accepted from other scientists throughout the world.

The papers must be written in English and must be clear, concise, and styled for *Cereal Chemistry*.

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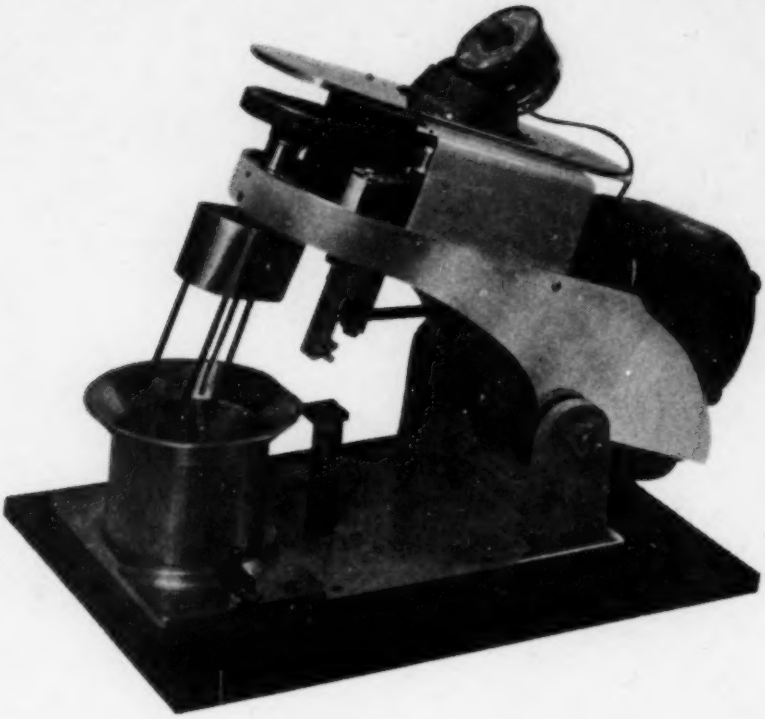

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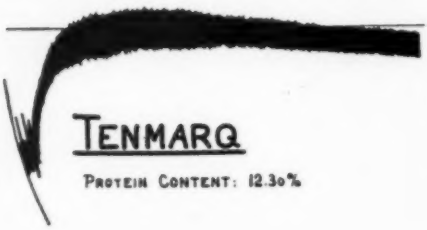

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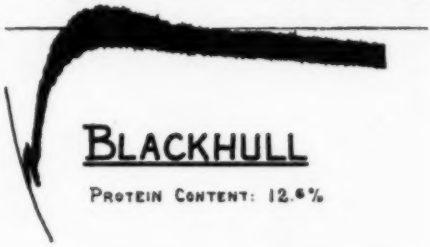

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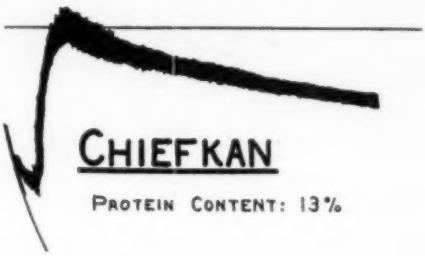

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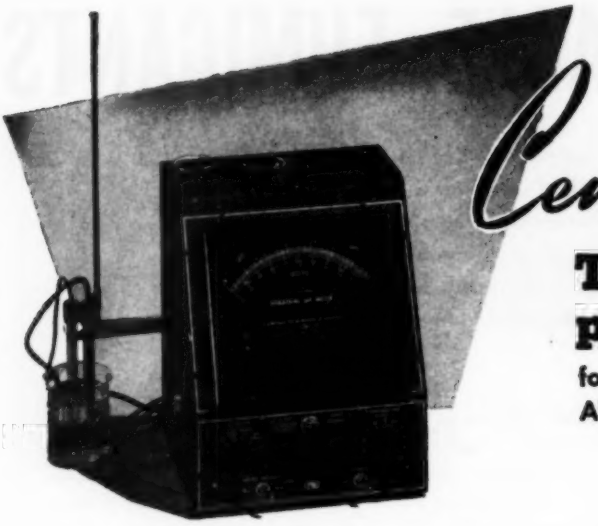

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CEREAL CHEMISTRY

VOL. XXV MARCH, 1948 No. 2

HYDROLYTIC TREATMENT OF SOYBEAN PROTEIN WITH PAPAIN [1]

VERNON L. JOHNSEN and ALLAN K. SMITH [2]

ABSTRACT

A study has been made of the effect of papain on the solubility of soybean protein determined at its isoelectric point. The substrate system was the water extract of soybean meal. While the pH of maximum activity was found to be about 8.0, there was good activity in the region of pH 5.0 to 9.0. Proteolysis proceeded rapidly without the use of activators. Hydrogen peroxide, sodium peroxide, and sodium hypochlorite were good deactivators for the system. Ninety-five per cent of the protein became water soluble at pH 4.2 in 3 hours at 60°C.

There are a number of food and industrial applications for soybean protein where various degrees of hydrolytic treatment are desirable, either for modifying the physical properties of the dispersed protein or for increasing its dispersibility in mild alkalies or water.

An outstanding example of protein modification by hydrolytic treatment is in the development by Urquart (12) and Perri (5, 6) of a foam stabilizer which served such a useful purpose during the war.

Smith and Max (11) studied the adhesive properties of soybean protein as they are affected by a mild hydrolytic treatment. They found that a protein prepared by simple alkaline extraction and acid precipitation has a low adhesive value if dispersed in alkaline salts, such as sodium carbonate or trisodium phosphate, but has a high adhesive value if dispersed in sodium hydroxide. In contrast to this behavior, the adhesive value of the protein in alkaline salts is greatly increased if the protein has received a mild hydrolytic treatment with sodium hydroxide during its preparation. This simple experiment illustrates a fundamental difference between soybean protein and casein, since the adhesive value for casein is not significantly different when dispersed in either mild or strong alkalies.

There are other applications where modification in dispersion characteristics resulting from mild hydrolytic treatment would be

[1] Manuscript received November 20, 1947.

[2] Northern Regional Research Laboratory, Peoria, Illinois, one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

expected to improve the product. Such applications include textile sizing, paper sizing, water emulsion paints, whipping agent, vegetable milk, and its use as a protective colloid.

If the desired hydrolytic treatment with alkali or with acid is at room temperature, it requires either considerable reagent or much time. Conducting the hydrolytic treatment at elevated temperatures would shorten the time factor but would bring other difficulties into operation, especially control of the reaction. The present investigation was undertaken to explore the possibilities of shortening this time factor by carrying out a mild hydrolytic treatment with an enzyme. The enzyme papain, described by Balls, Thompson, and Jones (1), was selected because it is a strong proteinase, relatively abundant, and relatively cheap. The present investigation is limited to a survey of the factors known to have the greatest influence on proteolytic action, such as pH, temperature, concentration of the enzyme, and the use of activators and deactivators. For the enzymatic process to be commercially feasible, it must be precisely controlled, and a method must be available for complete deactivation at a given stage of the reaction.

Materials and Methods

The protein system used in this investigation was a water extract of hexane-extracted soybean meal and contained, along with the protein, the sugars, phosphatides, salts, and other water-soluble constituents of the soybean meal. The protein in the system constituted less than half of the total solubles. The water extract of the meal had a pH of 6.6 to 6.7. It was anticipated that the behavior of such a system might be different from that of a solution containing only protein. The solution was prepared by stirring flakes with 12 times their weight of water and then removing the undissolved meal in a centrifuge. Such a dispersion contained about 1.8% protein of which 79.5% was precipitable with acid at pH 4.2.

The enzyme used was a water extract of commercial whole Ceylon papain. In making up the enzyme solution, the crude papain was ground in a mortar with a small amount of water, then diluted to 40 parts of water, mechanically shaken for 20 minutes, and centrifuged to remove the insoluble residue. The enzyme solution was further clarified by filtration. It had a pH value of 5.3.

Several different samples of crude papain were analyzed and found to contain 9.6% to 10.1% nitrogen, of which 94% to 96% was dispersible in water at a ratio of 1 to 40. However, one batch of enzyme was used for nearly all the work reported here. The reproducibility of the method of preparing the enzyme was found to be satisfactory by comparing the activity of several samples. The activity of the enzyme

was checked at intervals by measuring its action on gelatin by the Van Slyke amino nitrogen method. The experimental points on the curves in Figs. 1 to 5 are the average results of two or more experiments. The data in Tables I and II are the results of a single experiment.

Method of Measuring Hydrolysis. The measurement of hydrolysis of soybean protein in the volumetric Van Slyke apparatus was impractical because of the insolubility of the protein under the conditions of measurement. Other methods were tried and the one found to be the most satisfactory gave the change in the protein solubility at its isoelectric point of pH 4.2 as determined by Smith and Circle (8, 9) and Circle and Smith (2) with proteolysis. After a given reaction period, the enzyme was inactivated, the pH adjusted to 4.2, the dispersion centrifuged, and the nitrogen in the centrifugate determined. The increase in soluble nitrogen over that in the unhydrolyzed protein was taken as a measure of hydrolysis. The change in solubility of the protein with enzyme hydrolysis was much greater in the early than in the later stage of the reaction. However, for the present study the early stage is the more important part of the reaction and by any method of measurement the results are only relative.

Results

Effect of pH on Papain Activity. Hoover and Kokes (4) recently studied the effect of pH upon proteolysis of casein by papain. From their work and that of others it is evident the effect of pH on enzyme activity varies somewhat with the substrate. It was necessary, therefore, to survey the effect of pH on the soybean protein system and to find the region of good enzyme activity as well as the regions of inactivity. It was considered possible that the reaction could be controlled, in practical application, through pH adjustments.

A problem arising from the "change in solubility" method, which requires special consideration in the measurement of effect of the pH on activity, is the influence of buffer salts for maintaining constant pH on the dispersibility of the protein. The present work is directed toward a practical application where the use of added buffers could not be economically justified. Fortunately, these added buffers have been unnecessary for satisfactory pH control. At 25°C. and without buffers, the greatest pH change (lowering) of the system occurred in the region of pH 4.0 to 8.0 and for the first 2 hours amounted to less than 0.2 pH units. A pH lowering, superimposed on that of the enzyme action, in the range of 6.5 to 8.5, and at about 40°C., is caused by the action of microorganisms on sugars. Without addition of enzymes this change may amount to 1.5 to 2.0 pH units in 20 hours. Smith and

Max (10) isolated protein from similar systems after they had been subjected to the souring action of bacteria and yeast for 20 hours at about 40°C. They found no appreciable difference in the amount of nitrogen precipitated by acids before and after the action of the souring microorganisms. When the reaction is carried out at 26°C. for a short period the effect of bacteria and enzyme on pH is greatly minimized. It will also be noted from the pH activity curve (Fig. 1) that activity does not vary greatly in the range of 6.0 to 8.0, thereby further minimizing the need for precise buffer control to obtain practical results in this region. At pH values of 11.0 and 1.7, the inactive regions for the enzyme system as well as little bacterial action, the pH change, without buffers, was no more than 0.2 unit for 20 hours.

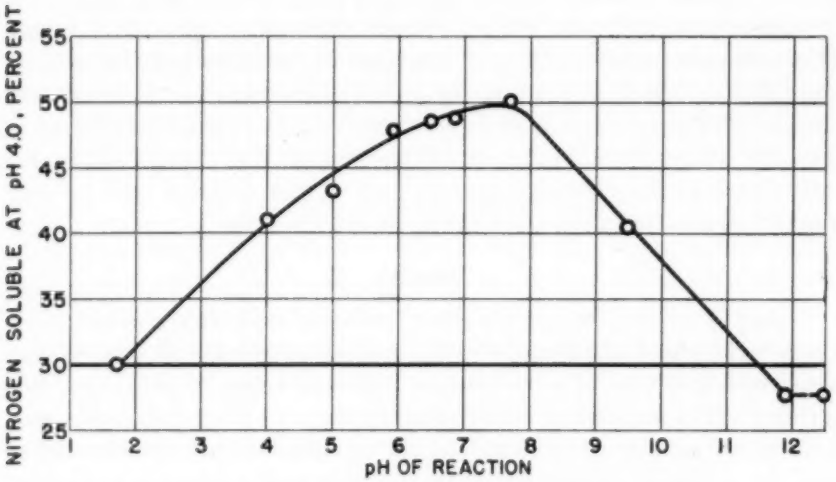

Fig. 1. The effect of pH on the hydrolysis of soybean protein with papain at 26°C. and E:S nitrogen ratio of 1:15. Reaction time 1 hour.

The salts added to the system (sodium sulfate) during neutralization procedures, as well as buffer salts, cause an increase in the protein dispersed; therefore, the salt concentration of the system was kept constant by adding salt solution as necessary. The total salt added amounted to 1.2 g. per 100 ml. solution. In the pH activity data, no correction has been made for the added nitrogen of the crude enzyme. These two factors, added salt and enzyme, give a constant increase of about 9.5% of soluble nitrogen in the system.

The results of the effect of pH on enzyme activity, for a one-hour period, are shown in Fig. 1. For these data the nitrogen ratio of crude enzyme to substrate was 1 to 15. The recorded pH values on this curve are the average of readings taken a few minutes after the addition of the enzyme and again at the end of the one-hour period.

From the results shown in Fig. 1, it may be concluded that the maximum activity is at about pH 8.0; nevertheless, there is good activity throughout the range of 5.0 to 9.0. Separate experiments demonstrated that the enzyme was undergoing inactivation at pH 1.7 and lost activity beyond recovery in 65 minutes at 25°C.

When the enzyme solution (pH 5.3) was added to the water extract of the meal (pH 6.6) a light precipitate was formed, possibly a complex between the protein and enzyme due to widely different isoelectric values. This precipitate disappeared when salts were added or the pH of the system was raised to about 7.5. If the pH of the system

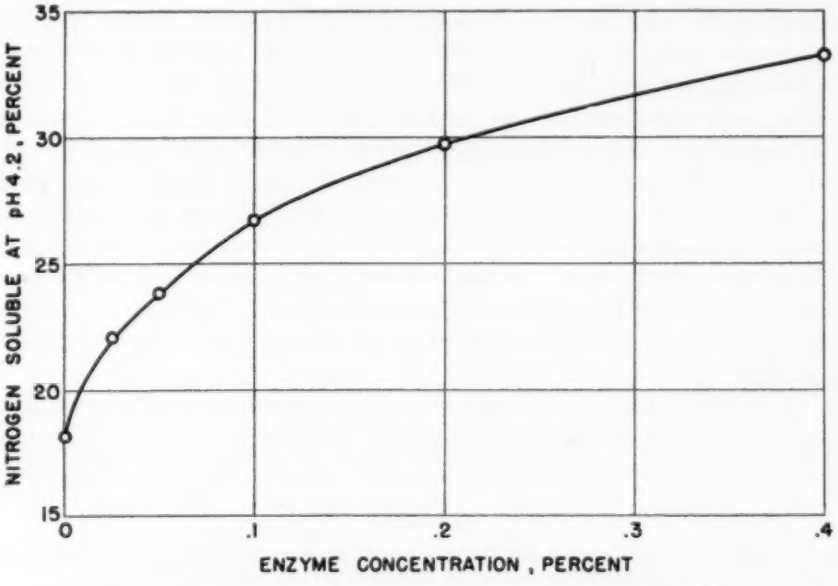

Fig. 2. The relation of concentration of crude enzyme to the change in nitrogen solubility at pH 4.2. Reaction period of 60 minutes, at 26°C., and pH 8. Enzyme concentration based on total weight of solution.

was lowered below 6.6, the precipitate increased. The formation of this precipitate is believed not to be a pH effect on the soybean protein dispersion, as without added enzyme the dispersion shows no precipitation until the pH is lowered below 5.5. It appears probable that the precipitate formed on addition of the papain to the protein is a factor, along with the pH, in reducing enzyme activity in the region below pH 8.0.

Enzyme Concentration. The studies on pH activity were carried out at an enzyme-substrate nitrogen ratio of about 1:15. Further studies were conducted to determine the effectiveness of papain hydrolysis at other enzyme concentrations. The proteolysis was carried

out for 60 minutes at 26°C., with a pH value of 8.0 and without added activators. The results are shown in Fig. 2.

While there is no section of the curve showing a strictly direct variation with enzyme concentration, nevertheless such a relationship is closely approximated in the range of 0.025% to 0.1% of enzyme. The remainder of the work included an enzyme concentration of 0.05% on the weight of the dispersion which is equivalent to an enzyme-substrate nitrogen ratio of about 1:60 or proportionately one-fourth of that used in the pH activity studies.

Activators and Deactivators. It was found that activators, which are customarily required for efficient papain activity, had relatively little effect on the proteolysis as it occurred in the water extract of the soybean meal, and the reaction proceeded efficiently without the addition of reducing agents. The effective proteolytic results obtained without added activators should not be unexpected in view of the work of Gottschall (3). In studies on hydrolytic treatment of beef, liver, and near beer, Gottschall found that apparently the activator is supplied by the exposed −SH groups of the protein and that as proteolysis proceeds the rate of reaction increases because of increased concentration of the −SH groups. While no attempt was made to check the concentration of −SH groups in soybean protein systems, the explanation presented by Gottschall concerning the source of activator may be applicable.

According to Scott and Sandstrom (7) the kind and concentration of activator greatly influence the relative activity of the papain, and as activator concentration increases, its effect passes through a maximum.

TABLE I

DATA SHOWING THE ACTIVATING EFFECT OF HYDROGEN SULFIDE AND THIOPHENOL ON PROTEOLYSIS OF SOYBEAN PROTEIN AT 26° AND 40°C. ONE HOUR REACTION TIME, 30 MG. CRUDE PAPAIN PER 75 G. OF PROTEIN DISPERSION AT PH 8.0 [1]

H_2S saturation %	At 26°C.			At 40°C.				
	Total soluble nitrogen %	Increase in sol. nitrogen[3] %	Mg. thiophenol per 100 g. of dispersion	Total soluble nitrogen %	Increase in sol. nitrogen[3] %	H_2S saturation %	Total soluble nitrogen %	Increase in sol. nitrogen[3] %
0	24.3[2]	—	0	23.7[2]	—	0	33.3[2]	—
0.01	25.8	1.5	0.08	24.2	0.5	0.01	33.4	0.1
0.12	25.8	1.5	0.80	25.5	1.8	0.12	34.0	0.7
1.20	26.4	2.1	8.00	30.8	7.1	1.20	35.1	1.8
12.0	27.5	3.2	80.00	28.5	4.8	12.00	36.5	3.2
100	31.3	7.0	800.00	31.8	8.1	100.00	39.7	6.4

[1] Soluble nitrogen in the original solution was 21.5%.
[2] The amount of soluble nitrogen in the system without added activator.
[3] As a result of the added activator.

In view of their results, the activating effect of hydrogen sulfide and thiophenol was studied at several concentrations. The results of activator studies are shown in Table I. The data in Table I and also in Figs. 1 to 5, inclusive, demonstrate that added activators are unnecessary for a rapid rate of proteolysis for the present system.

It is a fortunate circumstance that activators are unnecessary for the present system, since those normally used are undesirable in a system intended for industrial or food applications. For the two activators investigated there was no indication of the maximum effect demonstrated by Scott and Sandstrom (7).

Hydrogen peroxide, sodium peroxide, and sodium hypochlorite were examined as deactivators. These oxidizing agents were added from 5% solutions to 75 ml. of water extract of the soybean meal. The pH was adjusted to 7.0 and enzyme added at an E:S nitrogen ratio of 1:15. The system was digested at room temperature for 2 hours. The protein precipitated at pH 4.2, and a portion of the whey was analyzed for nitrogen. The results are shown in Table II in terms of percentage of total nitrogen of the solution appearing in the whey.

TABLE II

EFFECT OF DEACTIVATORS ON PAPAIN ACTIVITY

Ml. of 5% deactivator	Nitrogen in whey[1]		
	H_2O_2 %	Na_2O_2 %	NaOCl %
0[2]	22.1	21.5	21.8
0.1	37.5	35.3	38.6
0.3	27.6	31.3	41.5
1.0	22.2	25.2	24.1
3.0	23.3	23.9	16.6
5.0	22.8	24.4	21.3

[1] On the basis of total nitrogen in original dispersion.
[2] Blank determination, no enzyme or deactivator.

From these data it appears that approximately 1 ml. of a 5% solution of deactivator is sufficient to stop the reaction. This amounts to about 1 part deactivator to 4 parts crude enzyme. A more exacting technique would be required to measure precisely the relative effectiveness of deactivators. Hydrogen peroxide is the most satisfactory of these deactivators, since the sodium peroxide produced a very stable foam in the alkaline region and the hypochlorite discolored the isolated protein.

Fig. 3 shows additional data on deactivation with hydrogen peroxide when an excess of the deactivator was used at the same E:S ratio as in Table II and under various temperature conditions. Curve 5

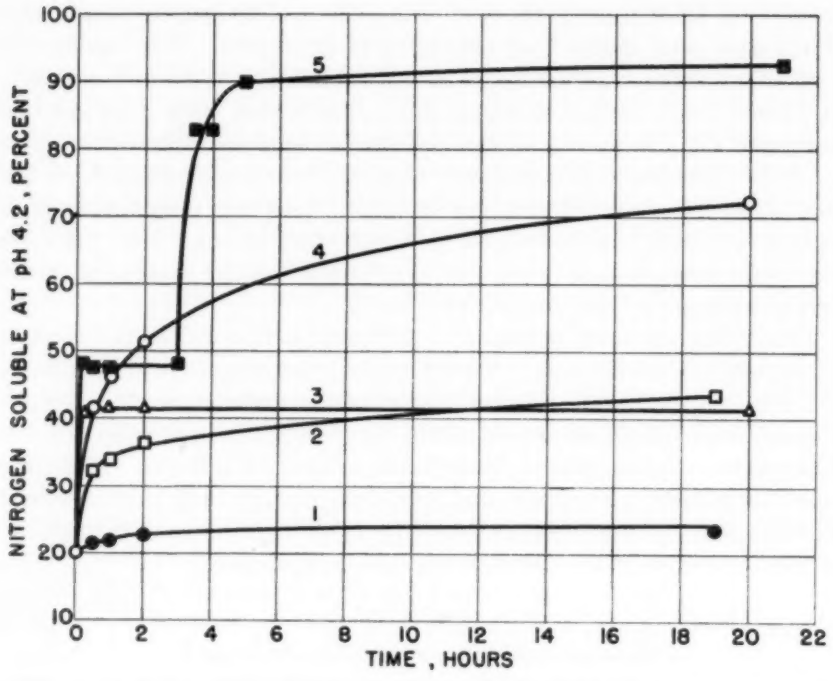

Fig. 3. The effect of hydrogen peroxide as a deactivator at several different temperatures. Curve 1 at 26°C. with hydrogen peroxide added to protein solution before the enzyme. Curve 3 at 40°C. with the hydrogen peroxide added 15 minutes after the enzyme. Curves 2 and 4 are reactions at 26°C. and 40°C. without deactivator. Curve 5 at 60°C. is an example of reversible deactivation with hydrogen peroxide added 5 minutes after the enzyme and sodium sulfite 3 hours later.

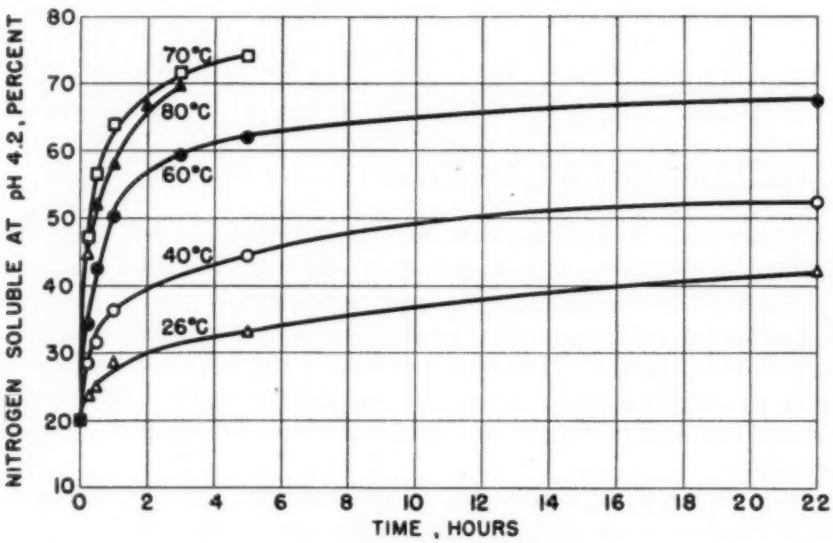

Fig. 4. The effect of temperature on rate of reaction at E:S nitrogen ratio of 1:60 and at pH 8.

of Fig. 3 is an excellent example of reversible deactivation. The hydrogen peroxide was added 5 minutes after the reaction started and brought it to an abrupt halt; 3 hours later the peroxide was destroyed with sodium sulfite and the reaction proceeded at normal or near normal rate as shown by comparing it with curve 5, Fig. 5. This latter reaction is the same as that shown in curve 5, Fig. 3, but without interruption of the hydrogen peroxide and reactivation with the sodium sulfite. When a correction is made for the 3 hours lost due to the hydrogen peroxide, the two curves are very nearly the same.

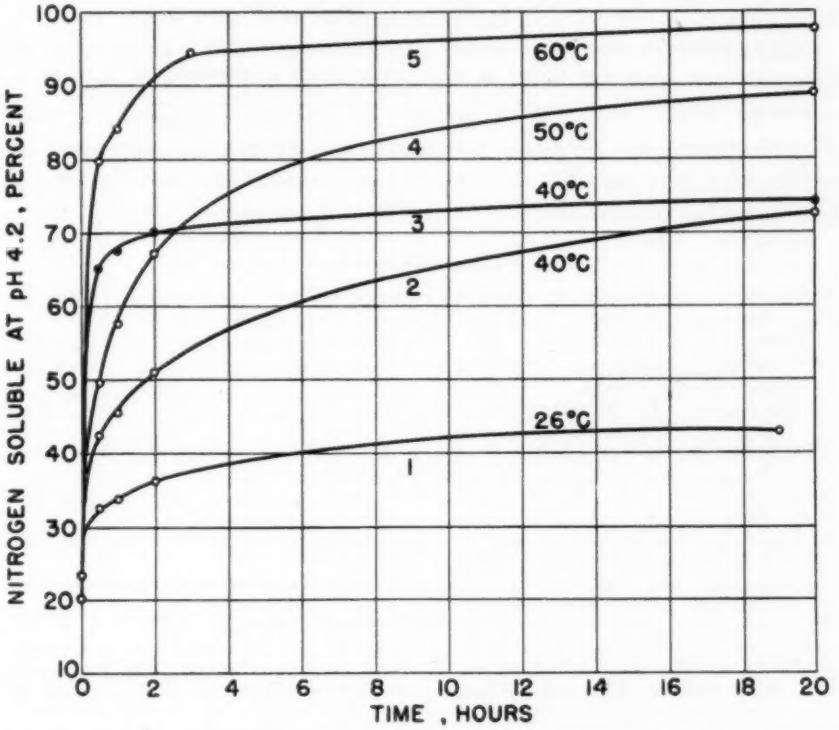

Fig. 5. The effect of temperature on rate of reaction at E:S nitrogen ratio of 1:15 and at pH 6.6. Curve 3, the protein solution, was boiled 5 minutes prior to the hydrolysis. The higher of the two zero values is for the curves at 50° and 60°C.

In Fig. 3, curves 2 and 4 represent reactions at 26° and 40°C., respectively, without deactivators; in curve 1 the hydrogen peroxide was added to the protein before the enzyme and in curve 3, 15 minutes after the enzyme.

Effect of Temperature. The data on effect of temperature on papain activity are shown in Fig. 4 for E:S nitrogen ratio of 1:60 and in Fig. 5 for E:S nitrogen ratio of 1:15. In these experiments no activator was used and the crude enzyme was added to the protein dis-

persion after it had been adjusted to the indicated temperature. At the time intervals shown in Fig. 5 a sample was withdrawn from the system, the enzyme deactivated with hydrogen peroxide, and the nitrogen precipitated at pH 4.2. The results show a very rapid increase in proteolysis with increase in temperature. In Fig. 5, for example, the raising of the temperature from 26°C. to 60°C., and for a 2-hour reaction time, the increase in soluble nitrogen amounts to 55%. Curve 3, Fig. 5, shows the reaction on a protein sample previously boiled for 5 minutes as compared to an unboiled sample shown in curve 2. The difference between these two reactions is largely in the early stages.

The enzyme activity is slowly destroyed at 80°C. In another experiment not recorded here, it was found that at 95°C. it required between 15 and 30 minutes to destroy the enzyme completely. However, in considering the rate of destruction of an enzyme with heat, the results may be somewhat different for an isolated enzyme than for an enzyme substrate mixture.

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STUDIES ON BREAD STALING. III. INFLUENCE OF FERMENTATION VARIABLES ON BREAD STALING AS MEASURED BY COMPRESSIBILITY AND FARINOGRAPH PROCEDURES [1]

J. FREILICH [2]

ABSTRACT

The crumb compressibility and the farinograph consistency procedures were used simultaneously in studying the effects of several fermentation variables and related factors on the staling of bread. The procedures are described and their relative accuracy compared.

The factors studied were variations in amount of yeast at constant dough volume and at constant fermentation time, variations in fermentation time of straight doughs and sponge doughs, and the effects of a crumb-softening agent.

No really significant effects on the rate of staling were produced by the fermentation variables so far studied, under the conditions used in this work.

Compressibility and farinograph data showed satisfactory agreement on the effects of fermentation variables on the rate of staling. There was some disagreement on apparent freshness. Where large differences in volume were involved, only the compressibility values indicated comparable differences in softness, which is said to be one criterion of freshness or consumer preference. There was definite disagreement on the effects of a crumb-softening agent; the compressibility procedure showed that this agent produced a definite decrease in the rate of staling, while the farinograph procedure showed a slight tendency in the opposite direction.

It is suggested that both procedures are of value in the study of bread staling, and that they be used simultaneously in continued studies on this problem.

The Committee on Food Research, of the Quartermaster Food and Container Institute for the Armed Forces, has been investigating the general problem of staling in bread. As a part of this work, the Fleischmann Laboratories have been conducting a study on the effects of type and extent of fermentation on the rate of staling.

Since there is no generally accepted method for measuring bread staling, it appeared advisable to compare available procedures for this purpose, among which are the crumb compressibility and the farinograph consistency procedures.

Several questions came to mind in this connection. To what extent do these procedures agree? If they don't agree, which is preferable? Which is more accurate? Which gives better correlation with consumer preference?

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The subject matter of this paper has been undertaken in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

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Preliminary work had indicated some disagreement between the compressibility and farinograph consistency procedures. It was therefore decided to use both procedures simultaneously in studying the effects of some fermentation variables on the rate of staling.

Materials and Methods

Baking Procedure. The following formula was used:

Flour	100% (1800 g.)
Water	64 to 66%
Yeast (variable)	2%
Sugar	5%
Diamalt	1%
Salt	2%
Shortening	3%
Milk powder	3%
Arkady	0.25%

Six loaf sponge or dough batches were mixed in the Readco mixer. Amounts of ingredients were based upon standard size (1 pound) loaves.

The sponges (60% of the flour, 37% water, the yeast, and the Arkady) were fermented at 30°C. for 3½ hours, then mixed with the balance of the dough ingredients at 30°C.; after 30 minutes the dough was divided into six equal portions, which were rounded, molded by machine after a 15-minute rest period, proofed to the top of the pan at 40°C., and baked at 215°C. for 30 minutes.

The straight doughs were fermented for about 2 hours (to a dough volume of 1180 ml. for a one-loaf dough), divided into six equal portions, rounded, molded 15 minutes later, then proofed and baked as in the sponge-dough procedure.

Two of the loaves were used for farinograph consistency measurements, and the remaining four loaves for compressibility measurements.

Compressibility Method. Changes in compressibility of bread are used as a measure of the rate of staling, since stale bread is much less compressible than fresh bread. Compressibility was measured by noting the extent of penetration of a plunger (1.9 cm. in diameter, with a spherical end) into a cube of bread crumb under a fixed stress and standard conditions, as described below. The type of apparatus used is a modified rough balance, described by Platt (3). A cube of bread measuring about 1½ inches thick and 2½ inches square is placed on a flat surface; the counterbalanced plunger is brought down so it just touches the surface of the bread, and a movable scale marked in millimeters is placed behind the balance pointer at the zero reading. Stress is exerted on the plunger by slowly admitting 100 ml. of water through a small opening into a container resting on top of the plunger,

which has been counterbalanced with it. The water flows into the container in 20 seconds, and extent of penetration is measured 3 minutes later. The first reading is made on the fresh bread 1½ hours after removal from the oven; readings thereafter are made at about 24, 48, and 72 hours.

Results representing averages of readings on four replicate loaves are expressed as compressibility, in millimeters, and as change in compressibility, in per cent; the first reading is taken as 100% compressibility. During the test, the bread is kept in double wax paper to avoid excessive loss of moisture. The bread is stored at room temperature.

Farinograph Method. The farinograph procedure used is based on one originally suggested by Fuller (1), and recently developed by Geddes and collaborators (2). The method makes use of the fact that the consistency of bread crumb and water "doughs," at constant moisture, decreases as bread undergoes staling changes.

A small farinograph bowl (50 g. capacity) was used to measure changes in maximum farinograph consistency of bread crumb and water "doughs" having a total weight of 80 g. and a moisture content of 60%. A portion of the loaf to be measured was cut off, and the rest of the loaf wrapped in double wax paper and saved for future measurements. The crumb was removed from the cut portion, and shredded in the Waring Blendor; the moisture of the shredded crumb was determined, and farinograph curves made with amounts of crumb and water calculated to make a "dough" of 60% moisture. Farinograph curves were made when the bread was one hour out of the oven, and about 24, 48, and 72 hours thereafter. Since the moisture determination required about 2 hours, the exact moisture content was not known at the time the fresh (one-hour) bread was tested; approximate amounts of crumb and water, at two levels in the region of 60% moisture, were therefore used, and a farinograph consistency value calculated for 60% from the two observed values, after the moisture of the crumb was determined.

Tests were made in duplicate, and results recorded as farinograph consistency, and per cent change in farinograph consistency, with the original value on the one-hour bread taken as 100%.

Results

Accuracy of Compressibility and Farinograph Procedures. The relative accuracy of the two procedures is indicated in Table I, which shows the mean values and standard errors obtained by both procedures on loaves from the same dough batch. In these experiments, doughs with 0.5, 1, and 2% yeast were subjected to constant fermentation

TABLE I

COMPRESSIBILITY AND FARINOGRAPH CONSISTENCY DATA FOR STRAIGHT DOUGH BREAD WITH DIFFERENT AMOUNTS OF YEAST AND CONSTANT FERMENTATION TIME

Amt. of yeast (%)	Age of bread (days)											
	0			1			2			3		
	COMPRESSIBILITY (MM.)											
	Mean	S	Per cent error	Mean	S	Per cent error	Mean	S	Per cent error	Mean	S	Per cent error
0.5	9.1	0.46	5.1	4.6	0.224	4.9	4.3	0.283	6.6	3.2	0.152	4.7
1.0	14.1	1.88	13.3	7.4	0.69	9.3	5.2	0.10	1.9	5.0	0.14	2.8
2.0	21.8	1.17	5.4	10.4	0.35	3.4	8.3	0.19	2.3	7.5	0.27	3.6
	FARINOGRAPH CONSISTENCY (B.U.) AT 60% MOISTURE											
0.5	—[1]			230	0	0	212.5	3.5	1.7	195	0	0
1.0				245	7.07	2.9	235	7.07	3.0	205	0	0
2.0				275	7.07	2.6	245	7.07	2.9	225	7.07	3.1

[1] The farinograph readings on the fresh bread (0 days) were not averaged, because they were obtained at different moisture levels, in order to serve as a basis for calculating a value for 60% moisture (see farinograph method in text).

NOTE: S = Standard error (or standard deviation, as defined in *Cereal Laboratory Methods*, 5th Ed., 1947). Per cent error = $\frac{S}{\text{mean}}$. B.U. = Brabender Units. Each compressibility mean represents four individual readings and each farinograph mean represents two individual readings.

time. Each compressibility value represents four individual readings, and each farinograph value represents two individual readings.

The compressibility readings on the fresh bread (0 days) usually show the greatest errors, or deviations from the mean values. The standard errors for 0.5, 1, and 2% yeast at 0 days (Table I) were, respectively, 0.46, 1.88, and 1.17 mm., or 5%, 13%, and 5% of the mean values. Thirteen per cent is, of course, a large error; in this set of readings the maximum error was much greater than the standard error; if the one reading which showed this large error had been discarded, the standard error would have been greatly reduced. Errors of a similar order of magnitude are not infrequent in compressibility measurements, however, and it is therefore important to keep this in mind in interpreting compressibility data.

The compressibility values for 1% yeast at 1 day showed a standard error of 9%; this was the largest deviation found outside of the readings for the fresh bread (0 days). Comparable deviations for the farinograph consistency values were very much smaller (Table I).

The farinograph readings for fresh bread (Table I) could not be averaged because they were obtained at different moisture levels. An experiment was therefore conducted in which six replicate loaves were used in farinograph measurements at constant moisture (60%) when the bread was 3 to 3½ hours old, and the crumb moisture had been determined. For comparative purposes, portions of the same loaves were also used in compressibility measurements. The results are shown in Table II.

TABLE II

INDIVIDUAL COMPRESSIBILITY AND FARINOGRAPH CONSISTENCY READINGS ON SIX REPLICATE LOAVES OF FRESH BREAD MADE FROM STRAIGHT DOUGH [1]

Loaf no.	Compressibility (mm.)	Farinograph consistency[2] (B.U.)
1	17.5	440
2	18.5	420
3	18.1	420
4	18.3	420
5	18.7	435
6	18.5	425
Mean	18.3	427
Standard error (S)	0.43	8.75
Per cent error $\left(\frac{S}{\text{mean}}\right) \times 100$	2.35	2.05

[1] Age of bread, 3 to 3½ hours.
[2] Farinograph consistency at 60% moisture.

The standard error for the farinograph values was 8.75 B. U. or 2.05%. Surprisingly, the standard error for the compressibility values was 0.43 mm. or 2.35%, an unusually accurate result, considering the errors generally encountered in compressibility data.

From all the above data, it seems apparent that the farinograph procedure is inherently more accurate than the compressibility procedure. It has been observed, however, that the accuracy of the compressibility procedure is susceptible of improvement with experience. If the technician is careful to avoid placing the plunger immediately above large holes or hard lumps on the crumb surface, the errors involved may be distinctly minimized. The cube of bread crumb should be cut after the measurement to observe the possible presence of large holes under the surface which were not visible when the test was made.

Effects of Varying Amounts of Yeast in Doughs Fermented to Constant Volume. The results of tests with 0.5, 1, and 2% yeast in straight doughs fermented to constant volume are shown in Fig. 1. The findings by both procedures may be outlined as follows:

Compressibility	*Farinograph Consistency*
2% yeast produced the highest values, or best apparent freshness.	2% yeast produced the highest farinograph values, or best apparent freshness.
The effects of yeast on compressibility were irregular.	The effects of yeast on farinograph consistency were progressive (with amount of yeast).
The amount of yeast used did not change the rate of staling (per cent change in compressibility).	The amount of yeast used did not change the rate of staling.

The two procedures here showed satisfactory agreement on two important points: (1) apparent freshness and (2) rate of staling. They did not agree in that compressibility values showed an irregular trend, whereas the farinograph values showed a progressive trend with increasing amounts of yeast; this seems to be a point in favor of the farinograph procedure.

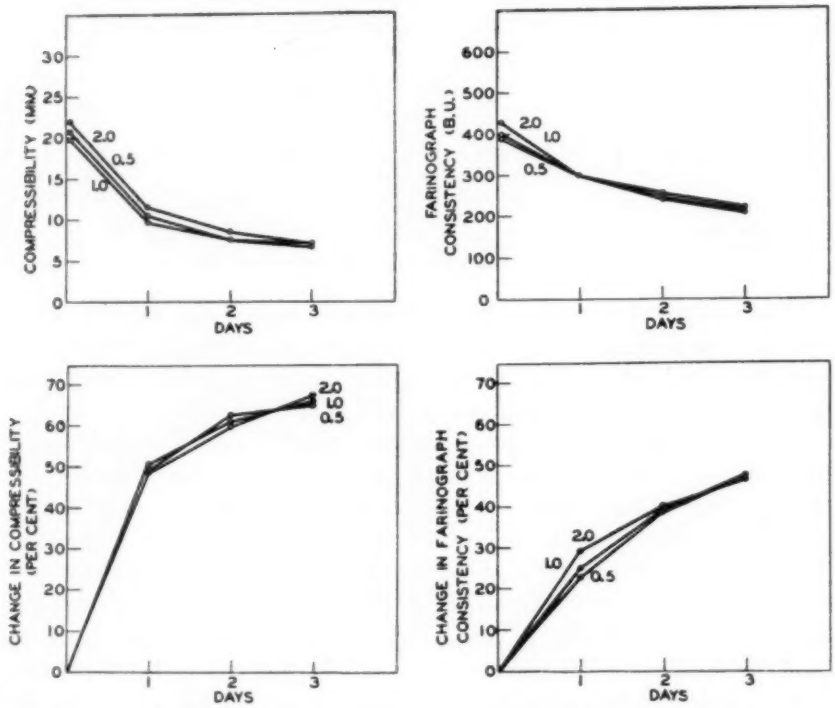

Fig. 1. Effects of 0.5, 1.0, and 2.0% yeast on compressibility and farinograph consistency of bread made from straight doughs fermented to constant volume.

Varying Amounts of Yeast in Doughs with the Same Fermentation Time. Fig. 2 shows the results of straight dough tests with 0.5, 1, and 2% yeast, all fermented for the same time, that which is normally

used for 2% yeast. The doughs with 0.5 and 1% yeast were, of course, greatly underfermented and underproofed; this was reflected in correspondingly poorer loaf volume, texture, and grain.

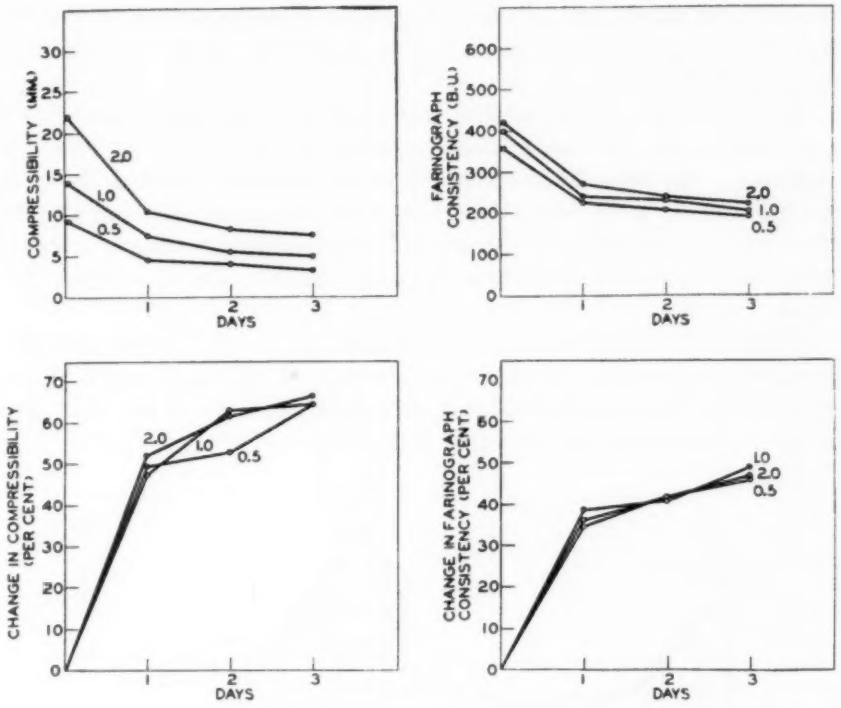

Fig. 2. Effects of 0.5, 1.0, and 2.0% yeast on compressibility and farinograph consistency of bread made from straight doughs fermented for the same time.

The relative findings were as follows:

Compressibility	*Farinograph Consistency*
The 2% bread was by far the most compressible, throughout the test period.	The 2% bread showed greater farinograph consistency values throughout the test period.
Compressibility varied as the amounts of yeast used.	Farinograph consistency varied as the amounts of yeast used.
The differences due to the amount of yeast used were very great.	The differences due to the amount of yeast used were slight by comparison with those shown by the compressibility values.
Staling rate differences were insignificant.	Staling rate differences were insignificant.

There was good agreement here, except for one rather important point, the degree of difference produced. The compressibility pro-

cedure showed great differences, in line with the variations in amount of yeast used, and with the resulting differences in volume and texture; but the differences in farinograph consistency were relatively slight. Since compressibility is a direct measure of softness in bread, which in turn is an important criterion of consumer preference, it is obvious that the compressibility procedure is preferable as an indicator of apparent freshness.

However, the compressibility values in this experiment serve as a good illustration of how misleading softness may be as an index of freshness. We know that the 0.5% bread was just about as fresh as the 2% bread, since both were baked on the same day (about 1½ hours apart), and yet, on the basis of compressibility values or softness alone, the 2% bread would be considered much fresher than the 0.5% bread.

Effects of Variations in Fermentation Time of Straight Doughs. Straight doughs with 2% yeast were fermented for ½, 2, and 4 hours, respectively, then proofed to normal volume (the top of the pan) and baked. The results are shown in Fig. 3.

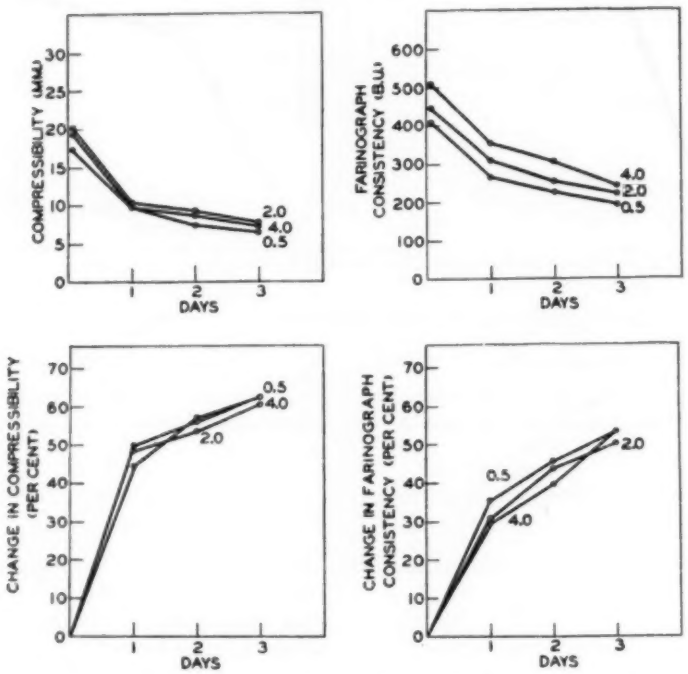

Fig. 3. Effects of 0.5, 2.0, and 4.0 hour dough times on compressibility and farinograph consistency of bread made from straight doughs.

The relative findings may be listed as follows:

Compressibility	*Farinograph Consistency*
The 2-hour fermentation produced the most compressible bread.	The 4-hour fermentation produced the highest farinograph consistency.
The differences due to fermentation time changes were irregular.	The differences due to fermentation time changes were progressive.
The 4-hour fermentation produced greater compressibility than the ½-hour fermentation, although the latter gave greater loaf volume.	The differences were relatively greater than those shown by the compressibility values.
There was no significant effect on the rate of staling.	The 4-hour fermentation produced a slight reduction in staling for the first 2 days, but there was no difference in rate at the end of 3 days.

The agreement between compressibility and farinograph consistency for these tests was not good. The compressibility values in-

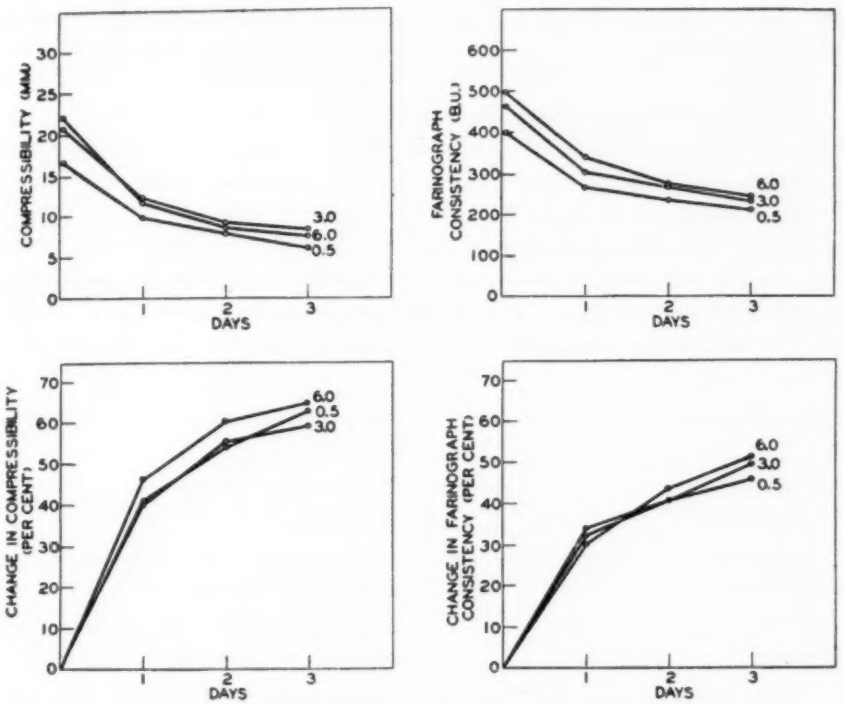

Fig. 4. Effects of 0.5, 3.0, and 6.0 hour sponge fermentation times on compressibility and farinograph consistency of the bread produced.

dicated the best apparent freshness for the 2-hour fermentation, which also produced the best loaf volume and bread quality, and would thus be preferable from the consumer viewpoint. On the other hand, the farinograph values indicated that the 4-hour fermentation produced

the best apparent freshness, and showed better correlation with fermentation changes throughout.

Effects of Variations in Sponge Fermentation Time. Sponges with 2% yeast were fermented for ½, 3, and 6 hours, respectively, then remixed, proofed, and baked as indicated in the procedure. The results are shown in Fig. 4.

The relative findings were as follows:

Compressibility	*Farinograph Consistency*
The 3 and 6 hour breads were the most compressible, with the 3 hour bread the better of the 2 except at 0 days.	The 6 and 3 hour breads produced the highest farinograph consistency values, with a slight advantage in favor of 6 hours throughout the test period.
The trend in compressibility values was irregular as regards sponge time, but varied as loaf volume.	The trend in farinograph values was progressive with respect to sponge time.
The 6-hour fermentation produced a slight increase in the rate of staling during the first 2 days.	The 6-hour fermentation increased the rate of staling to a slight extent during the 2nd and 3rd days, as compared to the ½-hour fermentation.

The agreement between the two procedures was fair in these tests; both showed the beneficial effects of sponge fermentation on apparent freshness, and both showed a tendency for the 6-hour fermentation to increase staling to a slight though unimportant extent.

Effects of a Crumb-Softening Agent. A product recently marketed as a crumb softener[3] for yeast-raised goods was tested in sponge-doughs at levels of 0.5 and 1%. The results are given in Fig. 5.

The following were the relative findings by the two procedures:

Compressibility	*Farinograph Consistency*
The softener increased compressibility.	The softener decreased farinograph consistency.
The trend was progressive (except for the fresh bread).	The trend was progressive.
The differences in compressibility were fairly large.	The differences in farinograph consistency were distinct.
There was a significant decrease in rate of staling.	There was a slight though perhaps insignificant increase in the rate of staling.

These results showed a definite disagreement between the two procedures. There was a negative correlation between the effects of the softener, one procedure showing an increase, and the other a decrease in apparent freshness. The most important difference, however, was shown by the per cent change in the values obtained by both procedures. The compressibility values indicated a significant inhibition

[3] A polyoxyethylene stearate with an average chain length of nine oxyethylene residues per molecule.

in the rate of staling due to the softening agent, whereas an opposite though less significant trend was indicated by the farinograph values. Though both procedures show evidence of change, only the change in compressibility or softness would be discerned by the consumer. On the basis of consumer preference, therefore (assuming that softness is a valid criterion of preference), the compressibility procedure appears to have an important advantage over the farinograph procedure. However, the very fact that the farinograph data are in complete dis-

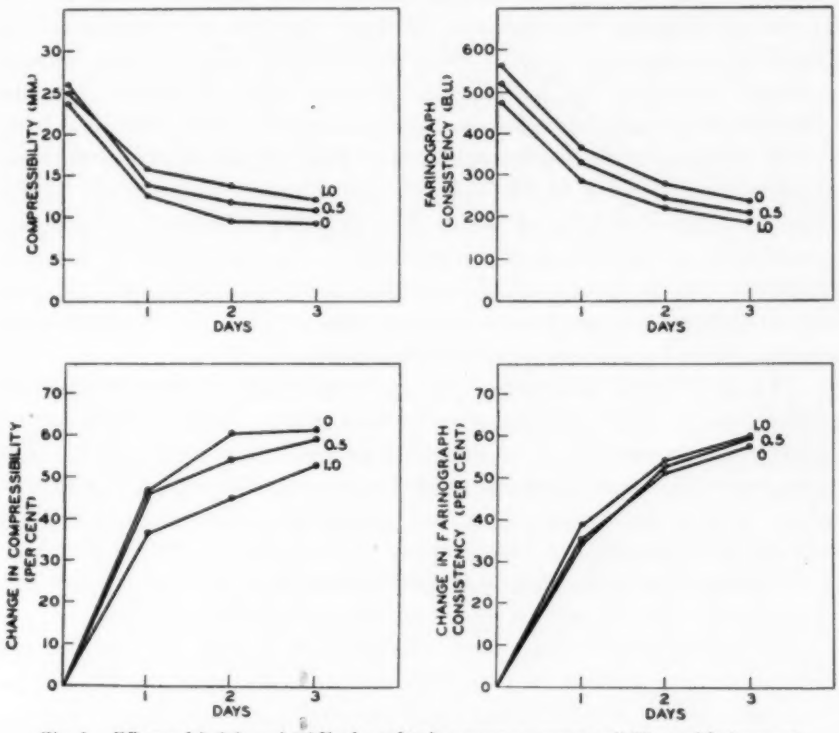

Fig. 5. Effects of 0, 0.5, and 1.0% of a softening agent on compressibility and farinograph consistency of sponge-dough bread.

agreement on this point is sufficient to cast some doubt on the true nature of the change indicated by the compressibility values.

Discussion

Compressibility and farinograph data were essentially in agreement on apparent freshness and rate of staling as influenced by several fermentation variables.

The following points of disagreement were noted: (1) In testing the effects of ½, 2, and 4-hour dough times, the best apparent freshness was obtained with the 2-hour dough time, according to com-

pressibility data, and with the 4-hour dough time by farinograph data. (2) The variables studied always produced progressive trends in the farinograph values, but the trends in compressibility values were irregular, and apparently were influenced by volume and texture. (3) The extent or degree of difference was much greater in the compressibility values when the volume differences were large. Where doughs with different amounts of yeast were fermented for the same time, the larger loaves were much more compressible or softer; the farinograph differences for similar loaves were relatively slight. (4) There was definite disagreement between the two procedures on the effects of a softening agent. Here the compressibility values showed increased softness and a definitely lowered rate of staling, but the farinograph values showed decreased consistency and a slightly higher staling rate due to the softening agent. The validity of crumb softness alone as a criterion of freshness is, of course, open to question. Variations in volume or type of bread may produce differences in softness when there is no difference in freshness. Flavor is also a factor in freshness. It is quite conceivable that a heavy, compact loaf may retain volatile flavors longer because less surface for evaporation is exposed than in a larger but otherwise similar loaf.

It is indicated that there was not enough agreement between the procedures to justify the use of either one alone. There was, however, sufficient agreement to show that both procedures were of value. The compressibility procedure showed better correlation with softness, which is one aspect of consumer preference. The compressibility procedure is also less time consuming than the farinograph procedure. The farinograph procedure is more accurate, and produced more uniform trends with respect to the effects of the variables studied.

Perhaps the most important consideration is that both procedures measure changes which are progressive in time, and of a roughly similar order of magnitude; these changes are probably related to true staling. If a factor is found which inhibits staling significantly by both procedures, one might be fairly certain that true rather than apparent freshness is involved.

Acknowledgment

The author wishes to express his thanks to R. F. Light, Manager, and G. W. Kirby, Technical Director, of the Fleischmann Laboratories, for their helpful interest, and to Miss Joan Bachman and Harold Wagner for their valued assistance in this work.

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CHEMICAL STUDY OF THE MATURE WHEAT KERNEL BY MEANS OF THE MICROSCOPE [1]

M. R. SHETLAR [2]

ABSTRACT

The composition of the wheat kernel was investigated by microchemical methods *in situ*. Cellulose is present in large amounts in the walls of the epidermal, cross layer, and hyaline cells. Some is present in the walls of the aleurone cells, but only a small amount occurs in the testa layer. Lignin occurs in the middle lamella of the cross layer cells, in some of the epidermal cell walls, and in the wheat hairs. A layer of cutin is present on the epidermis of the wheat kernel, and the testa cell walls are heavily cutinized. Lipid material is present within the aleurone cells. Pectic compounds appeared to be present in all of the bran layers, being located either in the middle lamellae or in the cell walls adjacent to them. Protein material was found largely in the testa, aleurone cell contents, and starchy endosperm, being especially high in the aleurone layer.

As the structure of the wheat kernel is of considerable practical importance to the agronomist and to the milling technologist, the microscopic appearance of the wheat kernel has been extensively studied and reviewed (3, 4, 5, 6, 11). However, aside from several studies of the development of the wheat kernel (1, 2, 7, 9), microchemical methods were not extensively employed. It is the purpose of this paper to present the results of a preliminary microchemical study *in situ* of the mature wheat kernel.

Materials and Methods

A sample of Ohio soft red winter wheat and a sample of Michigan white wheat were used to furnish kernels for the work. Cross and longitudinal sections were made by soaking the grain for about 4 hours in water and then sectioning it with the freezing microtome. Sections of approximately 20μ in thickness proved to be most satisfactory. Flat sections of the various bran layers were obtained either by stripping them by hand from the kernel or by chemical separation as previously described (10).

Photomicrographs were originally made in natural color using a Leitz photomicrographic camera with a special adapter for 35 mm. film, and a Bausch and Lomb polarizing microscope. Photographs presented here are black and white transfers made from the original kodachrome transparencies.

[1] Manuscript received July 14, 1947. Presented at the Annual Meeting, May, 1946.
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Microchemical tests used were those compiled by Sampson (8) and may be divided into four types: (1) tests depending upon differential staining, (2) tests depending upon specific color reactions, (3) tests based upon solubilities, and (4) tests based upon optical properties. The tests may also be classified as follows according to the constituent which is being studied.

Cellulose. A blue or violet color reaction occurs when tissue containing cellulose is placed in the chloro-zinc-iodide reagent (25 g. zinc chloride, 8 g. potassium iodide, 1.5 g. iodine, 8 ml. water). Cellulose lamellae are soluble in ammoniacal cupric hydroxide (ammonium hydroxide is added to saturated copper sulfate solution until solution appears green, then filtered; the precipitate is washed with water, dried, and dissolved in ammonium hydroxide solution). Cellulose lamellae are also soluble in 75% sulfuric acid solution. Cellulose lamellae are anisotropic under crossed nicols, usually with positive elongation when observed in cross and longitudinal sections of tissue.

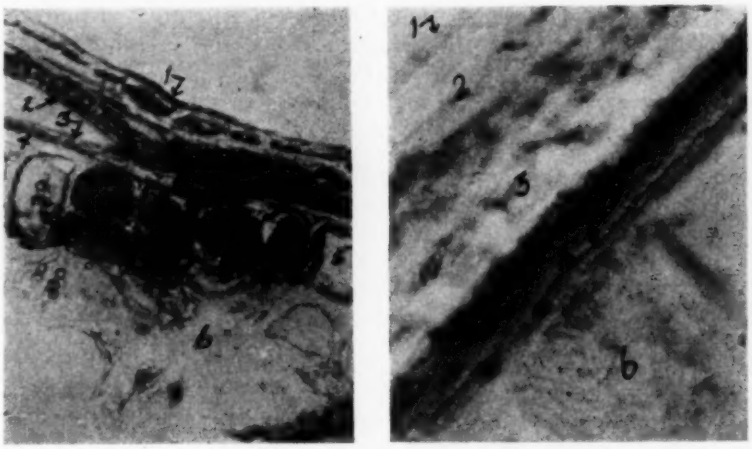
Cutin and Suberin. Tissue containing either cutin or suberin are stained by Sudan IV (0.1 g. Sudan IV, 5 ml. 95% ethanol, and 5 ml. glycerol) when placed in the dye solution for some time and then washed with 50% ethanol. If sulfuric acid is added to tissue thus stained, the stained areas become blue. Cutin and suberin are soluble in a 10% solution of potassium hydroxide in either ethanol or glycerol after heating for one hour. Droplets of ceric acid appear in sections containing cutin or suberin upon treatment with Schultze's reagent (equal volumes of nitric acid and potassium chlorate solution) followed by gentle heating. Suberin is distinguished from cutin by the potassium phellonate test which consists of macerating the tissue with saturated potassium hydroxide solution for several hours, heating mildly, and finally heating to a boil. The potassium phellonate appears as granular masses which give a violet-red color reaction with the chloro-zinc-iodide reagent. Suberin lamellae are anisotropic, while pure cutin lamellae are isotropic; however cutinized cellulose lamellae are anisotropic with negative elongation.

Pectic Compounds. Pectic compounds may be differentially stained by placing a section in ruthenium red solution (one part ruthenium red to 10,000 parts of aqueous weakly ammoniacal solution) for about 20 minutes, followed by thorough washing with water. All pectic compounds are soluble when heated with dilute acids (2% hydrochloric acid solution) for 30 to 60 minutes and then treated with 2% potassium hydroxide solution. Pectic lamellae, cutinized pectic lamellae, and lignified pectic lamellae are isotropic under crossed nicols.

Pentosans. Tissues containing pentosans undergo a red to violet color reaction when a section is placed in a solution of phloroglucin

(1% in ethanol) for a few minutes and then is placed in a drop of concentrated hydrochloric acid and heated gently for 10 minutes.

Lignin. A red color reaction is obtained without heating with the phloroglucin-hydrochloric acid test as described for pentosans when lignin is present. Lignified pectic lamellae are isotropic under crossed nicols, while lignified cellulose lamellae are anisotropic with positive elongation.



A B

Fig. 1. A. Photomicrograph of untreated cross section of wheat. 1. Epidermis; 2. Cross Layer; 3. Testa; 4. Hyaline; 5. Aleurone; 6. Starchy Endosperm. × 200.

B. Longitudinal section of wheat under polarizing microscope using selenite red plate. Black and white transfer was made using blue filter. Under these conditions outer part of epidermis (1) which is isotropic appears dark. Rest of epidermis which is blue in kodachrome print appears as more or less light area (2). Cross layers (3) likewise appear as light area. Testa (4) also being isotropic appears dark. Hyaline layer (5) which is blue in kodachrome print appears beneath testa layer. Part of an aleurone cell (6) is visible. × 900.

Proteins. The xanthoproteic test was used by placing the section in a drop of nitric acid solution (3–1 in water), drawing off the excess acid and adding a drop of ammonium hydroxide solution. Tissue containing protein becomes yellow to brown in color. Proteinaceous material in sections placed in a saturated aqueous solution of picric acid for some time gives a typical yellow reaction. The biuret test for protein may be used by placing sections in 5% cupric sulfate solution for 30 minutes, washing, and adding saturated potassium hydroxide solution. The protein material is stained a red to blue color.

Results

In all of the following, the various layers of the bran and the adjacent endosperm are labeled according to the nomenclature given in Fig. 1A.

Optical Properties. In cross or longitudinal sections of the wheat kernel, the outer layer of material of the epidermis was isotropic, being invisible under crossed nicols or red if the selenite red plate was used (Fig. 1B). The rest of the cell walls of the epidermis were anisotropic and appeared blue when placed parallel to the plane of vibration of the selenite red plate in both cross and longitudinal sections. When viewed in a flat section, the majority of the cell walls at the butt and brush ends of the epidermis had a negative elongation, appearing yellow when parallel to the plane of vibration of the selenite red plate. On the other hand, most of the cell walls of the center portion of the epi-

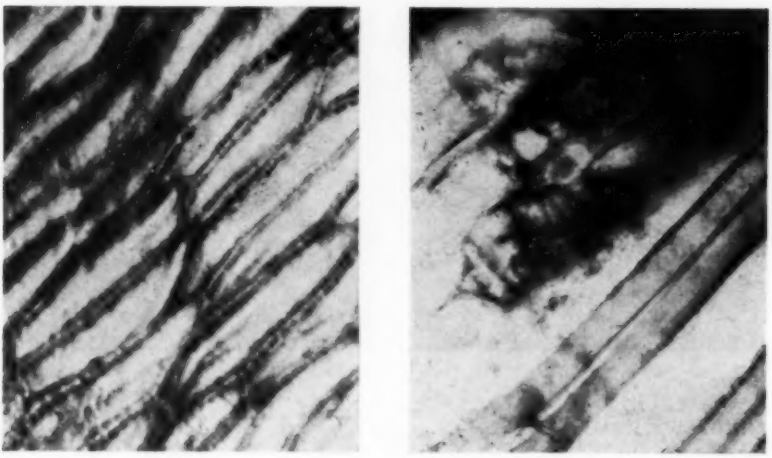


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Fig. 2. A. Photomicrograph of flat section of epidermis under crossed nicols using selenite red plate. Long axes of cells are parallel to the plane of vibration of selenite red plate. Dark portions are blue. × 200.

B. Section of wheat hair under crossed nicols parallel to the section of vibration of selenite red plate. Dark portions are blue. × 900.

dermis had a positive elongation. After treatment with the glycerol-potassium hydroxide reagent the middle lamella of the epidermis cells was swollen and appeared isotropic, and most of the remaining cell walls exhibited positive elongation (Fig. 2A). When placed parallel to the plane of vibration of the selenite red plate, the cell walls of the wheat hairs appeared blue under crossed nicols (Fig. 2B).

The walls of the cross layer cells were anisotropic under crossed nicols. When viewed parallel to the plane of vibration of the selenite red plate, they appeared yellow in a cross section of the wheat kernel, while in the longitudinal section they appeared blue under the same conditions. In a flat strip section (Fig. 3A) the inner walls of the cross layer cells appeared yellow, while the outer walls and middle lamella appeared purple when viewed parallel to the plane of vibration of the

selenite red plate. After treatment of these cells with potassium hydroxide in glycerol, all cell walls exhibited negative elongation. Treatment with ammoniacal cupric hydroxide solution destroyed the

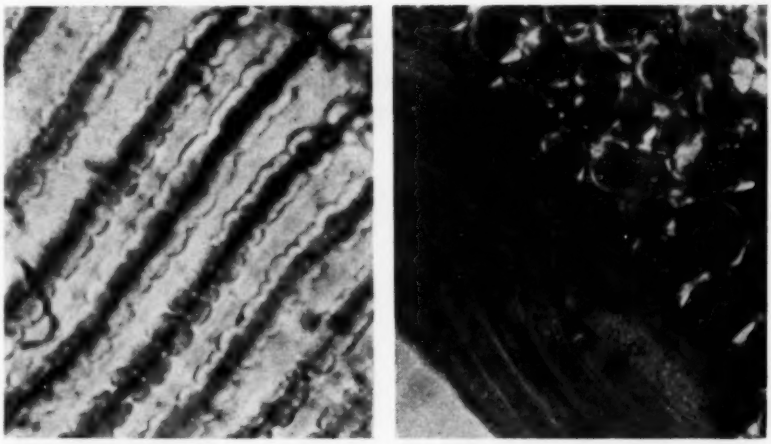

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Fig. 3. A. Photomicrograph of flat section of cross layer cells under crossed nicols. Long axes of cells are parallel to the plane of vibration of selenite red plate. Dark areas are purple; light areas are yellow. × 900.

B. Flat section of aleurone and hyaline cells under crossed nicols. Hyaline cells are the elongated cells to the left and have their long axes parallel to selenite red plate. Filled aleurone cells are in lower right hand corner; empty aleurone cells appear above them. Except for the aleurone cell contents which are naturally dark, the dark areas are blue and the light areas are yellow.

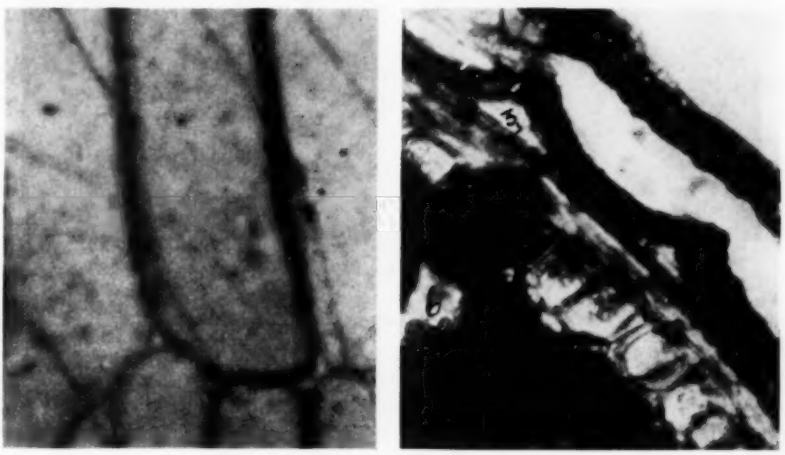

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Fig. 4. A. Photomicrograph of flat section of testa layers of white wheat after treatment with chloro-zinc-iodide reagent. Cell walls react faintly and appear dark in photograph. Outer layer of testa (in focus) is superimposed on inner layer (out of focus). × 900.

B. Photomicrograph of cross section of wheat after treatment with chloro-zinc-iodide reagent. Tissue containing cellulose becomes blue and appears dark in photograph. Starch also reacts so that starchy endosperm (6) also appears dark. Being brown, testa (3) appears dark although it did not react with reagent. × 200.

anisotropy of the cross layer cells. The cell walls of the tube cells had optical properties similar to those of the cross layer cells.

The walls of the testa cells were unique in that they were isotropic under crossed nicols.

The hyaline and aleurone cell walls were anisotropic (Fig. 3B). The aleurone cell contents were isotropic under crossed nicols. When viewed parallel to the plane of vibration of the selenite red plate, the cell walls of the hyaline cells appeared blue while the middle lamella

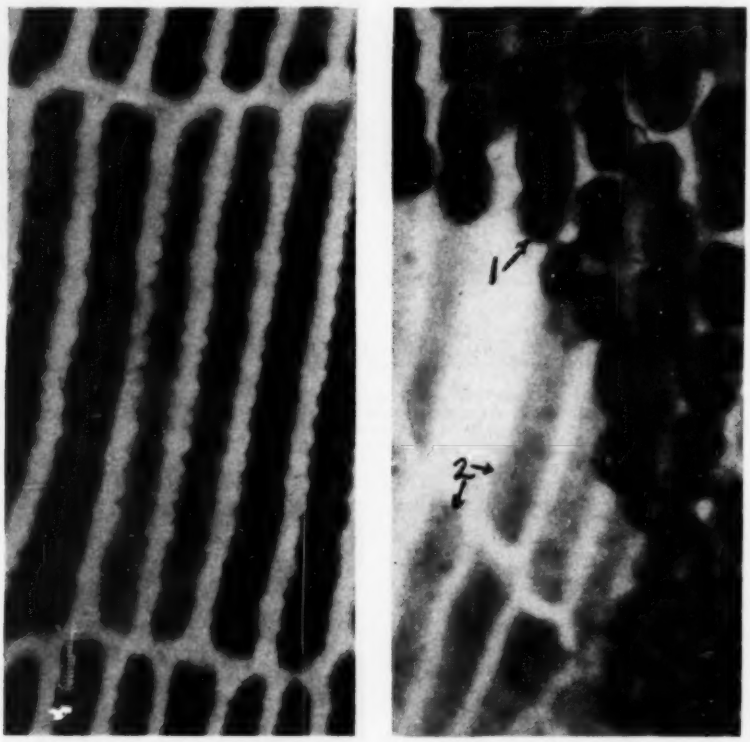

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Fig. 5. A. Photomicrograph of flat section of cross layer cells of wheat after treatment with chloro-zinc-iodide reagent. Tissues containing cellulose become blue and are dark in photograph. × 900.
B. Photomicrograph of flat section of aleurone cells (1) superimposed upon hyaline cells (2) after treatment with chloro-zinc-iodide reagent. × 200.

was yellow. Aleurone cell walls parallel to the selenite red plate also appeared blue.

The cell walls of the starchy endosperm were anisotropic and were blue when viewed parallel to the plane of vibration of the selenite red plate.

Cellulose. With the possible exception of the testa layer, cellulose occurs in generous amounts in the various bran layers, as shown by the

chloro-zinc-iodide test carried out either on the original preparation or after treatment with potassium hydroxide in glycerol. The testa of red wheat either failed to react with the reagent or the reaction is

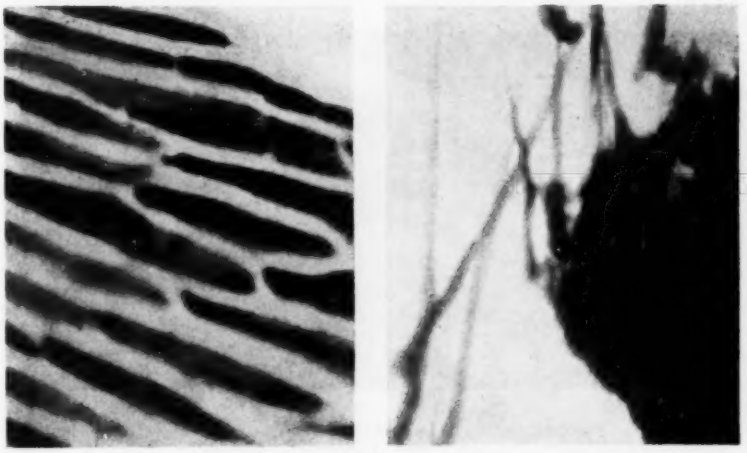

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Fig. 6. A. Photomicrograph of wheat epidermis flat section after treatment with glycerol-potassium hydroxide solution and then with chloro-zinc-iodide reagent. × 200.
B. Wheat hairs after treatment with chloro-zinc-iodide reagent. × 200.

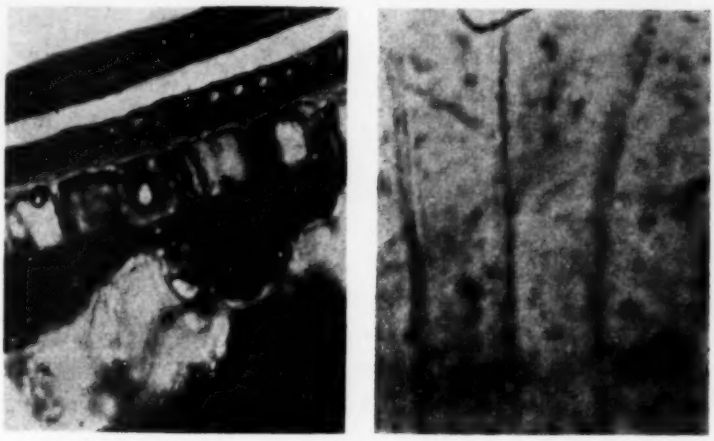

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Fig. 7. A. Photomicrograph of longitudinal section of wheat kernel after treatment with ruthenium red solution. Transfer was made with blue filter so that pink areas appear dark. × 200.
B. Flat section of white wheat testa after treatment with ruthenium red solution. Pink areas appear dark.

masked by the brown pigment of this layer. Strip sections of white wheat testa, however, reacted mildly with chloro-zinc-iodide (Fig. 4A). When a cross section of the wheat kernel was treated with the reagent,

the walls of the epidermis, the cross layer cells, the hyaline, and the aleurone cells gave a strong reaction (Fig. 4B). In strip sections the walls of the cross layer (Fig. 5A), the hyaline (Fig. 5B), and the aleurone (Fig. 5B) cells all reacted with chloro-zinc-iodide without

Fig. 8. Photomicrograph of flat section of cross layer cells after treatment with ruthenium red solution. Pink areas appear dark. × 900.

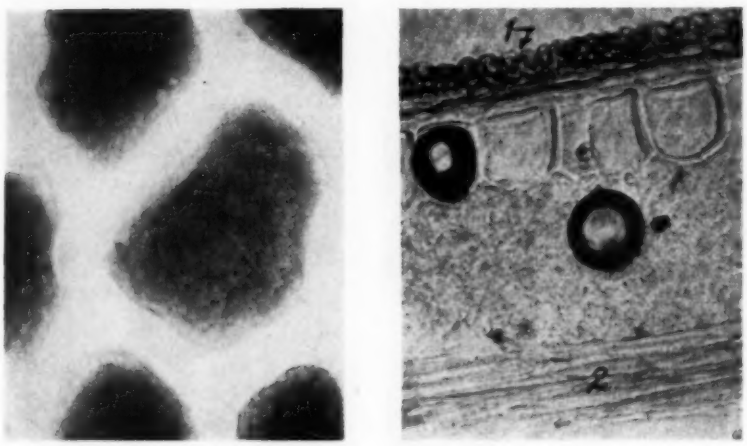

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Fig. 9. A. Photomicrograph of flat section of aleurone cells after treatment with ruthenium red solution. × 900.
B. Longitudinal section of wheat after treatment with phloroglucin and hydrochloric acid. Walls of cross layer cells (1) which contain lignin are stained red and appear dark. Epidermis which has washed away appears at 2. × 200.

preliminary treatment. After treatment with potassium hydroxide in glycerol, the walls of the epidermis cells (Fig. 6A) and the wheat hairs (Fig. 6B) reacted strongly with the reagent.

Pectic Compounds. Treatment of cross and longitudinal sections of the wheat kernel with ruthenium red indicated that pectic com-

pounds occurred in the walls of all of the bran layers (Fig. 7A). The cell walls of the epidermis layers in flat strip sections were stained with this reagent; however, after treatment with hot dilute hydrochloric acid and hot potassium hydroxide solutions the brush and butt ends of the epidermis still stained. The walls of the cross layer and the tube cells were stained by ruthenium red (Fig. 8). The walls of white wheat testa cells (Fig. 7B) and the middle lamellae of the hyaline and aleurone cells stained faintly with this reagent. The interior of the aleurone cells stained strongly (Fig. 9A).

Lignin. As indicated by the pink color reaction given by the phloroglucin-hydrochloric acid reagent when the test is applied to

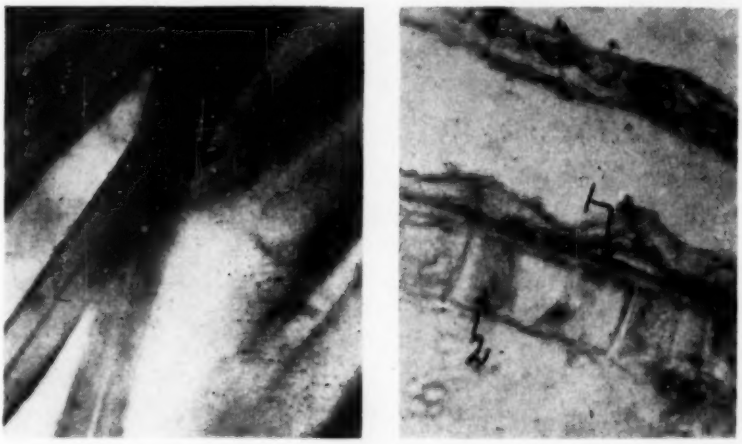

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Fig. 10. A. Wheat hairs after treatment with phloroglucin and hydrochloric acid. Lignified areas are pink and appear dark. × 900.

B. Cross section of wheat after staining with Sudan IV. Testa (1) is apparently cutinized and is stained red, appearing dark in photograph. Aleurone cell contents (2) also stain due to their fat content. × 200.

cross and longitudinal sections of the wheat kernel, lignin occurs in the cross layer cells, particularly in that portion adjacent to the testa cells (Fig. 9B). This observation was verified by making the test on a flat strip section of cross cells in which lignin appeared to be located either in the outer part of the cell wall or in the middle lamella. Lignin also occurred in the walls of the wheat hairs (Fig. 10A) and in the epidermis at the butt end of the grain. Some samples of epidermis contained scattered areas of lignification other than at the ends of the kernel. These consisted of lignified walls of a small group of cells, isolated cells, or, rarely, partial lignification of the cell wall of an isolated cell.

Cutin and Suberin. Cutin occurred as a covering of the epidermis, as indicated in cross or longitudinal sections of the wheat kernel under

the polarizing microscope (Fig 1B). A positive ceric acid test was obtained upon epidermis in a strip section.

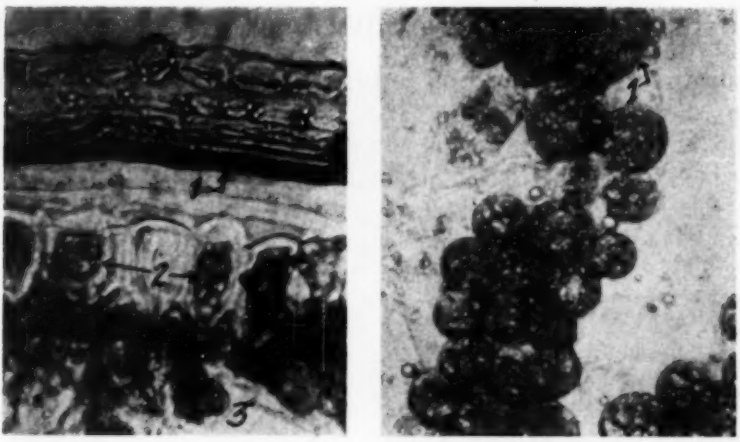

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Fig. 11. A. Cross section of wheat after execution of xanthoproteic test. Testa (1), aleurone cell contents (2), and starchy endosperm (3) all react strongly and appear dark in photograph. × 200.
B. Flat section of aleurone cells (1) superimposed upon hyaline cells after execution of xanthoproteic test. × 200.

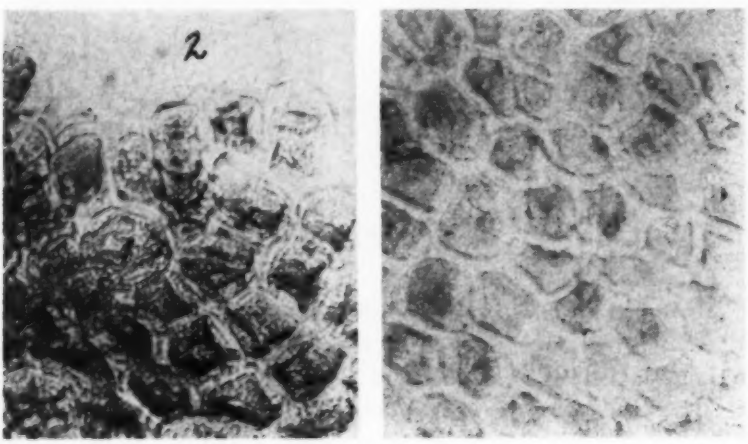

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Fig. 12. A. Photomicrograph of flat section of aleurone (1) superimposed upon hyaline cells (2) after applying picric acid test. Tissue containing protein is stained yellow and appears dark in photograph. × 200.
B. Flat section of aleurone to which biuret test has been applied. × 200.

Staining a cross section of the wheat kernel with Sudan IV indicated that the testa was either suberized or cutinized (Fig. 10B). With this dye the contents of the aleurone cells also stained very strongly. A strip section of testa removed by alcoholic sodium hydroxide solution

was also stained with Sudan IV. When a strip of whole bran was treated with 75% sulfuric acid solution for several minutes and then washed with water to remove the cellulose, pectin, pentosans, and proteins, the outlines of the testa cells could still be distinguished in the residue. This bran residue stained readily with Sudan IV and gave a positive ceric test. However, potassium phellonate tests were negative.

Proteins. As indicated by the xanthoproteic test on cross and longitudinal sections of white wheat, the proteins of bran occur largely in the testa layers (Fig. 11A). A small amount is found in the cross layers and the epidermis. A strip section of aleurone and hyaline, when treated with the xanthoproteic reagents, reacted only in the interior of the aleurone cells (Fig. 11B). Similar results were obtained using the picric acid test (Fig. 12A) and the biuret reaction (Fig. 12B). The starchy endosperm also gave a strong test for protein by all three methods.

Discussion

From the reactions with chloro-zinc-iodide it appears that cellulose occurs in abundance in the epidermis, the cross layers, the tube cells, and the hyaline cells. It is present to some extent in the cell walls of the aleurone, but is almost totally absent in the testa layer. These results are in accord with the quantitative work on wheat testa (10).

Since the cell walls of the epidermis after the removal of cutin are anisotropic with positive elongation and since this anisotropy is lost after treatment with ammoniacal cupric hydroxide solution, it appears that the cellulose of the epidermis is laid down parallel to the long axis of the cell. This is also true of the wheat hairs and of the hyaline cells. On the other hand, the inner cross layer cell walls exhibit negative elongation in the strip section and in the cross sections of the kernel. In a longitudinal section of the kernel in which the cross layer cells themselves are in cross section the cell walls exhibit positive elongation. Apparently then, the cellulose in the cross layer cells is laid down perpendicular to the long axis of the cells or parallel to the long axis of the grain. The difference in optical properties of the outer cell walls and middle lamellae of the cross layer cells as compared to the inner ones may indicate the presence of cutin in these tissues. This is further indicated by loss of these properties upon treatment with glycerol-potassium hydroxide.

The isotropy of the testa layer is striking since it has been reported that suberin lamellae are anisotropic while cutin lamellae are not, and other tests indicate that the testa contains either cutin or suberin. The testa layer is still isotropic after treatment with 75% sulfuric acid solution which removes everything except cutin, suberin, chitin, or

phytomelane. Although Eckerson (2) reported that the nucellus of wheat was suberized, her tests could hardly have distinguished between suberin and cutin. Since a potassium phellonic test was not obtained and since the testa layer was isotropic, it appears that this layer is cutinized rather than suberized.

The pectic compounds, as shown by staining with ruthenium red solution, were quite generally distributed in the bran. The staining of the epidermis with ruthenium red after treatment with weak hydrochloric acid and sodium hydroxide solutions was probably due to lignin, but no further attempt was made to learn whether pectic compounds also occurred in the same areas. The cross layer cells stained with ruthenium red in the cell walls next to the middle lamella, but not in the middle lamella itself. The middle lamella of these cells apparently contains lignin instead of pectic compounds. The cause of the staining of the aleurone cell contents with ruthenium red was not established.

Tests for protein indicated that little protein is found in any of the bran layers with the exception of the testa layer (Fig. 11A). This is in agreement with the quantitative data. The protein of the aleurone occurs almost entirely within the interior of the cell (Figs. 11B and 12).

Acknowledgment

The author is indebted to Dr. H. C. Sampson, Professor of Botany, Ohio State University, for his instruction and aid in the use of the microchemical methods employed in this work.

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STUDY OF THE WATER CONTENT OF SINGLE KERNELS OF WHEAT[1]

T. A. OXLEY[2]

ABSTRACT

Apparatus and technique are described which are suitable for the determination of the water content of single kernels of wheat in lots of 40 kernels at a time.

The standard deviation of each set of 40 water content results may be used to measure the range in water content. Wheat which has been stored in small containers (i.e., of the order of one liter or less) has a standard deviation in water content ranging from 0.13 to 0.41%.

Distinction is made between a range of water content due to kernels being in equilibrium with different humidities (the "unsteady state") and a range due to physical differences in the kernels giving differences in water content when all are in equilibrium with the same relative humidity (the "equilibrium state").

The range of water content is temporarily increased by exposure of wheat to very dry or damp atmospheres which lead to rapid drying or damping of the kernels. This is evidence that the kernels differ in the rate at which they are able to exchange water vapor with the atmosphere.

In one case investigated (using a sample of a soft white Scandinavian variety "Als"), rapid drying produced a skew distribution of water content. This is evidence of skew distribution among the kernels of a character which affects the rate at which they exchange water vapor with the atmosphere. There are indications that this is also true of some other varieties studied.

Wheats of mixed origin were generally found to have a wider water content range in the equilibrium state than wheats derived from a single crop.

In a single study of a soft red wheat (Squarehead Masters), ears during ripening were found in general to dry out first at the apex and last at the base, although a few kernels near the base of an ear dried as quickly as apical kernels. The range of water content within a single "dead ripe" ear was found to be large (standard deviation 0.74), but this was probably partly an unsteady state due to incomplete drying out.

The water content of a sample of grain may be determined with some precision, but since the kernels of the sample vary among themselves in size, shape, consistency, and degree of maturity at harvest, it is reasonable to expect that there may be differences in their water content even though they have lain together in the same container for a considerable period.

It was thought desirable to investigate the range of water content exhibited by grains of various types and histories for three reasons: (1) If the range of water content is known it is possible to evaluate the

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[2] Pest Infestation Laboratory, Department of Scientific and Industrial Research, Slough, England.

error in water content determination which will follow from this source of variation in samples of various sizes. (2) Study of the water content of single kernels provides an indirect method for investigation of the way in which individual kernels exchange water with the atmosphere. (3) It was thought that differences in water content between individual kernels might have an influence on the choice of grains for oviposition by grain weevils (*Calandra* spp.). No evidence bearing on the third point has been obtained during the present investigation.

Apparatus and Methods

The first investigations were carried out by crushing the kernels and drying them in small labeled glass or metal containers in the hot air oven which is used for normal determinations in the laboratory. This method was abandoned because it was excessively laborious and the water losses during crushing of the kernels were found to be quite serious. Instead of this a method was developed in which the kernels were not broken or cut but were dried whole.

Determination of water content on whole kernels has three distinct advantages: (1) There is no danger of loss of water vapor or material during crushing or grinding. (2) The labor of crushing or grinding (with the need for cleaning a small crushing or grinding apparatus between each kernel) is obviated. (3) The rate of water vapor exchange between whole kernels and the atmosphere is much slower than that of crushed or ground kernels. For this reason it is possible to weigh whole kernels, even when freshly dried, direct on the scale pan without appreciable error due to gain or loss of water vapor provided that the period of exposure does not exceed 30–40 seconds.

These advantages are very real in so far as they reduce labor, for a statistical study of the range of water content requires many determinations from each sample. The standard number of kernels determined per sample for the present investigation was 40 and the labor involved in these determinations would have been prohibitive if all laborsaving techniques had not been adopted.

The disadvantage of using whole kernels is that the rate of loss of the last traces of water is excessively slow. It was desired to use a temperature not greatly different from that which is standardized in this laboratory for normal water content determination (115°C.), and it was found that satisfactory results with whole kernels could only be obtained at or near this temperature, by use of a vacuum desiccator and a period of at least 48 hours. The apparatus described below was therefore constructed and, having adopted for convenience a standard drying time of 48 hours, the temperature was varied until agreement was obtained between the mean water content of 40 whole

kernels and the water content of a sample of grain from the same sealed bottle determined by the standard laboratory technique.[3] Agreement was satisfactory when the temperature of the surface on which the kernels lay was 120°–125°C.

Drying Apparatus. The arrangement of the vacuum desiccator used for drying 40 whole kernels simultaneously is shown in section in Fig. 1.

It was found very inconvenient to have a heater within the desiccator supplied by leads through the lid and accordingly a system of induction heating was adopted. A bundle of ¼-inch diameter soft iron rods stands vertically in the center of the desiccator and serves as a magnetic core in which an alternating magnetic field is induced by the exciting coil. The latter (which consists of 12 lbs. of copper wire 0.071 cm. diameter) is wound on a wooden former and fixed to the wooden stand in which the desiccator rests loosely.

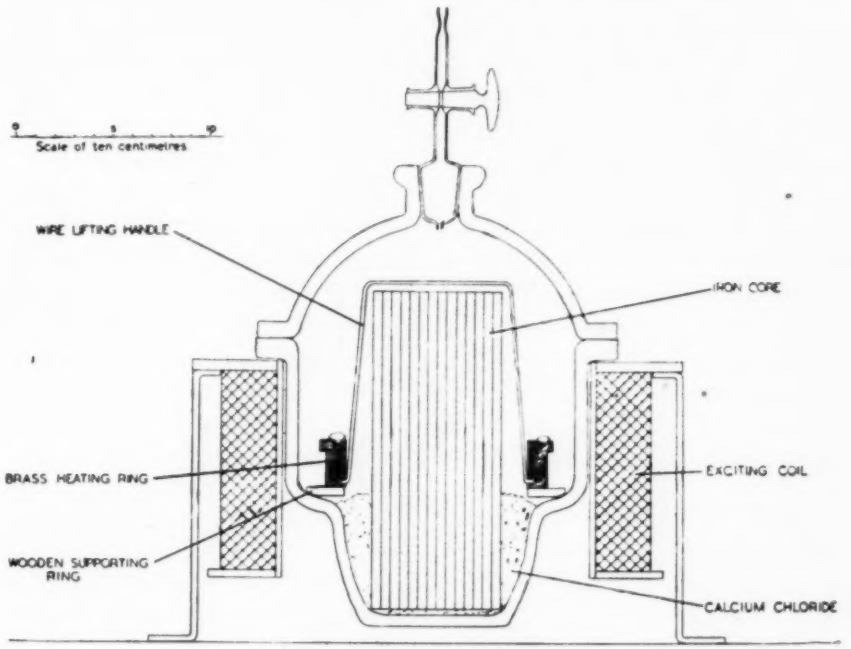

Fig. 1. Vertical median section through drying apparatus. Thermometer is not shown in the drawing.

The heater consists of a brass ring, of the form shown in Fig. 1, which surrounds the central iron core at about its mid height. The alternating current that is induced in this ring produces sufficient heat to raise it to the desired temperature. The amount of heat dissipated

[3] Grain coarsely ground and dried for 4 to 4½ hours in a ventilated oven at 115°C.

in this ring, and hence the temperature which it reaches, can be varied by varying the voltage applied to the exciting coil. This adjustment is made by means of a tapped auto-transformer not shown in the diagram. The voltage used is 322 at 50 cycles per second.

The temperature of the ring is indicated by a thermometer whose bulb rests in a hole bored vertically in the ring. Thermostatic control is not applied to the ring and hence the temperature reached depends partly on the temperature of the room in which it is placed. In practice the variations are slight (the extremes being of the order of ±5°C.) and no effect on the water content results has been observed. Even if slight variations of the mean water content determined by this method do occur they are unimportant for the present investigation, since the aim is to study interkernel variations rather than absolute water content values.

Forty shallow conical depressions about 7 mm. diameter and 1.5 mm. deep and numbered 1–40 are machined in the upper surface of the ring to receive the kernels for drying. The ring is provided with a wire handle (which runs parallel with the magnetic field and therefore does not become hot) and by this means may be conveniently transported to and from the balance room so as to receive kernels directly after weighing.

At the end of a drying period the lid of the drying chamber is removed and, while the ring is still hot, the kernels are transferred as quickly as possible to 40 small, numbered desiccators. Each of these consists of a glass tube 30 mm. long and 6 mm. internal diameter sealed at one end and closed with a rubber stopper at the other. A small amount of phosphorus pentoxide is put at the bottom of the tube and covered with a pad of cotton wool on which the kernel rests. Kernels cool rapidly in the desiccators and may be weighed immediately, but there is no gain in weight if they are left for several days before weighing.

In addition to the desiccators there are several sets of smaller rubber-stoppered glass bottles 15 mm. long by 4.5 mm. internal diameter which contain no drying agent. These are used for isolating sets of 40 kernels at various stages in the wetting and drying experiments or for collecting samples from various sources. Once isolated in these tubes kernels do not change in weight over periods of many days.

The Balance. It was desired to measure water contents of kernels with an accuracy of ±0.05%. Since the usual weight of kernels employed in the present investigation was between 20 and 50 mg., this required that weighings should be accurate to ±0.01 mg. It is not usually possible to combine this degree of accuracy with a rapidity

of operation such that the period of exposure of the kernels does not exceed 40 seconds. This difficulty was overcome by the use of a special balance designed and manufactured by L. Oertling and Sons, Ltd.

This consists of a microbalance with an optically projected scale for reading the last two decimal places such as is standard in this firm's normal commercial model. To this instrument the following modifications are made in order to satisfy the requirements of rapid weighing and accuracy specified above: (1) The sensitivity is reduced to one-tenth of the normal (i.e., full scale deflection represents 1 mg.). (2) Air damping is fitted so as to make the balance aperiodic. (3) Automatic loading is provided by means of four ring-riders. This enables loading by 10-mg. intervals from 0–90 mg. to be made by rotating a dial. (4) The usual 5 mg. rider is used only on every tenth notch of the notched beam, thereby giving loading from 0–9 mg. by 1-mg. intervals.

Judgment by eye of the weight of a kernel to within 10 mg. is easy. With practice it is possible to weigh most kernels with only one or two movements of the rider and the balance can thus be released within 15 seconds of the kernel being dropped on to the pan from its bottle or desiccator. The beam requires 15 seconds to come to rest after release and thus the whole weighing can be completed within 30–40 seconds.

Estimation of Errors. Since no independent check on the water content of a single kernel is possible, all errors appear as interkernel variations. Hence it is particularly important to examine the sources of error. There will probably be some variations in the extent to which the loss of weight on drying under particular conditions represents the "true" water content as it might be defined in physicochemical terms. But such variations, which may be called the "intrinsic" variations, are inherent in all known methods of water content determination and it is at present impossible to evaluate them. Hence, except in terms of an abstract physicochemical definition, it has no precise meaning to speak of a "true" water content. The term water content can thus only have definite meaning if the precise method of determination is specified.

The indirect loss-of-weight-on-drying method was adopted for the present investigation because it is the method most easily adapted to determination of single kernel water content. It is an advantage of the choice that similar methods are probably the most widespread throughout the world in laboratory and trade practice with cereals. The present results are therefore probably subject to the same intrinsic sources of error as all similar methods.

What may be called the extrinsic sources of error are those which arise from the technique, producing differences between the loss of weight on drying under constant conditions and the estimate of them. The possible sources of such errors in the present technique appear to be: (1) Human errors. (2) Variations in conditions between the numbered places occupied by the kernels during the course of the determination, i.e., variations in ring temperature from place to place and variation in humidity in the desiccators. (3) Errors in weighing.

It is believed that the technique leaves very little room for human errors to remain undetected. Unevenness in the temperature of the ring would produce an error, but such an error should be consistent over a long period since a difference in temperature between one part of the ring and other parts could only arise from a defect in the brass casting which caused local variations in electrical resistance. A similar consistent error would arise if any fault (such as exhaustion of the desiccant) existed in one of the single kernel desiccators. In order to ascertain whether any such consistent errors existed part of the data was examined in the following way.

A number of sets of water content determination records were chosen. For this purpose only sets having a standard deviation less than 0.3 were used since variability greater than this occurred only when the kernel had been subjected to a wetting or drying treatment in which cases it is clear that the excess variability is certainly in the material and is not characteristic of the technique. Thirty such sets were available. Thirty water content percentages obtained in each of the 40 positions were added together and the variance of the 40 totals was calculated and found to be 2.292. Therefore the variance between single determinations is estimated to be $\frac{2.292}{30 \times 30} = 0.00255$.

It has been assumed that this variance between single determinations is due solely to consistent inequalities in the technique related to the numerical position of the kernels. It is certainly not an underestimate and is probably an overestimate, since with so small a number as 30 sets some of the variance must be due to random distribution of variations in the material.

Errors in weighing are probably the most serious extrinsic source of variability. In order to estimate these, 40 kernels contained in 40 of the rubber-stoppered bottles described above were weighed seven times over a period of 9 days. In each case the kernels were taken in random order so as to avoid the danger that the operator might learn the weights. The weighings were thus conducted under precisely the same conditions as were the weighings for determination of water content.

This process provided seven estimates of the weight of each of the 40 kernels. For each kernel, the sum of squares of deviations from the mean was calculated. Allowing six degrees of freedom to this, the total sum of squares for the 40 kernels has 240 degrees of freedom. The variance of a single weighing calculated on this basis was found to be 0.000125 (all weighings in milligrams and decimals of a milligram).

The total weight of the 40 kernels showed a small progressive decrease which was first detectable on the fifth total. This was presumably due to slight drying out of the kernels during their successive exposures to the atmosphere during weighing. No allowance was made for this source of error in the above calculation of the weighing variance which is therefore presumably an overestimate.

The effect of the weighing error on the water content estimate varies according to the size of the kernel. If x = wet weight of the kernel in milligrams, and y = the dry weight,

$$\text{variance of } \frac{x-y}{x} = \frac{\text{var. } x + \text{var. } y}{x^2}$$

$$\text{but variance } x = \text{variance } y = 0.000125.$$

Therefore, variance of water content estimate: per cent of wet weight

$$= \frac{100^2}{x^2} \times 2(0.000125) = \frac{2.5}{x^2}$$

Therefore, if the weight of a kernel is as low as 15 mg. (which is the lowest extreme used in the present experiments) the variance in water content estimate due to weighing error is $\frac{2.5}{15 \times 15} = 0.011$. For heavier kernels it is clear that the variance due to this cause is very much less and is quite negligible by comparison with the variances of the water content estimates of the material.

In Table I are presented data from samples showing the minimum of variability, in which the weighing error and "place" error are of the greatest importance. In this table the variances due to these causes are given (that for weighing being calculated on the basis of the mean kernel weight) and in the last column the standard deviation which remains after these two variances have been subtracted is given. It will be seen that even these data are scarcely affected by these sources of error (compare columns 5 and 11). In all later data no allowance is made for errors since they are negligible by comparison with the variability of the material. It is clear that the variabilities studied in the present work are true variabilities of the material.

Results and Discussion

Differences in water content between kernels can be of two kinds: (1) Kernels may so differ physically that their water-holding capacities differ when in equilibrium with a given atmospheric relative humidity. This is referred to below as the "equilibrium state." (2) Kernels may be in equilibrium with different atmospheric relative humidities. This state of a sample is referred to below as the "unsteady state."

In the unsteady state the kernels are exerting different water vapor pressures, and if such kernels are contained in a common container their water contents will be unsteady; they will exchange water by diffusion until all kernels in the container exert the same vapor pressure. They will then be in equilibrium with the same relative humidity and the residual differences in water content will be of the first kind, i.e., the sample will be in the equilibrium state. The time required for such equilibration must depend very largely on the size of the container. No adequate data exist on this point, but some of the present data give evidence relating to very small containers.

Diversity in the Equilibrium State. Differences in water content in the equilibrium state appear to be distributed approximately according to a normal frequency distribution. Hence it is convenient to use the standard deviation of the results as a measure of the range of water content. Determinations have been made on a number of samples of wheat which, whatever their previous history, had been stored in the laboratory in small sealed bottles (about 200 ml.) or rubber-stoppered test tubes (about 30 ml.) for periods varying between a month and a year. It is assumed that relative humidity equilibrium had been completely attained in all these samples and that the variability remaining was due solely to physical differences in the kernels. The results of a series of such determinations are given in Table I.

It is clear from the data in Table I that there is an irreducible minimum of water content variation in wheat of various varieties when in equilibrium with the same relative humidity. The standard deviation of the various samples quoted is in the neighborhood of 0.2 based on samples of 40 kernels in each case.

It has not been possible to find any observable character in the individual kernels which could be correlated with their water content. On a number of occasions each kernel of a series has been carefully examined for such characteristics as color, wrinkledness of skin, length relative to breadth, plumpness, and freedom of the skin from breaks. No relation has been found between any of these and water content. There is also no sign of any correlation between water content and the weight of kernels taken from a sample in the equilibrium state. This fact is some evidence that the water content determination technique is

TABLE I

INTERKERNEL VARIABILITY OF WATER CONTENT IN SAMPLES OF VARIOUS VARIETIES OF WHEAT STORED IN SMALL QUANTITIES FOR ONE MONTH OR LONGER

Date	Variety	Water content mean	Gross variance	Standard deviation	Error correction			Total extrinsic variance	Residual	
					Mean kernel weight	Weighing variance	Place variance		Variance	Standard deviation
		%	%	%	*mg.*	%	%	%	%	%
24.4.46	Atle	18.15	0.048	0.219	37.18	0.0018	0.0026	0.0044	0.044	0.210
30.4.46	Unnamed white winter	15.03	0.058	0.240	48.65	0.0011	0.0026	0.0037	0.054	0.232
1.7.46	Als	18.33	0.037	0.193	47.81	0.0011	0.0026	0.0037	0.033	0.182
10.8.46	Als	20.55	0.049	0.222	46.45	0.0012	0.0026	0.0038	0.045	0.212
4.2.47	"Manitoba"	13.97	0.063	0.252	31.43	0.0025	0.0026	0.0051	0.058	0.241
8.2.47	Extra Kolben	13.61	0.022	0.150	34.06	0.0022	0.0026	0.0048	0.017	0.130
4.3.47	Meteor	13.42	0.043	0.207	34.41	0.0021	0.0026	0.0047	0.038	0.195
7.3.47	Bersee	12.95	0.169	0.411	52.14	0.0009	0.0026	0.0035	0.165	0.406

unaffected by kernel size, i.e., large and small kernels are dried equally effectively.

Effect of Recent Change in Water Content on the Interkernel Variation. It was observed that samples which had recently been dried by exposure to air of low humidity, or damped by exposure to air of high humidity, exhibited a wider diversity than those which had not been so treated. It was also observed that the increased diversity resulting from rapid gain or loss of water vapor disappeared during the course of a few days from a small sample confined in a sealed container, the diversity reverting to that characteristic of the equilibrium state. It was therefore concluded that the increased diversity was an unsteady state, the various kernels being in equilibrium with different humidities. It also follows that rapid drying or damping at ordinary temperatures does not affect the water-holding capacity of the kernels, since the diversity at equilibrium state was unchanged. The fact that diversity is increased by rapid change, however, shows that kernels differ in the ease with which they gain or lose water.

Kernel samples were dried by exposure for the stated period in a wire gauze basket in a desiccator containing calcium chloride and fitted with a fan in the lid. Samples were damped by spreading the kernels on a piece of perforated zinc held 1.5 mm. above a water surface in a petri dish. A filter paper soaked in water was fixed inside the lid about 8 mm. above the kernels. In the case of both drying and damping, the number of kernels seldom exceeded 200 and never formed a complete layer over the gauze or perforated zinc. It was considered

that all kernels had substantially equal exposure to the wet or dry atmosphere and hence substantially equal opportunities for water vapor exchange. The samples that were left for varying periods after treatment, before isolation in separate bottles, were always small, usually 100 to 150 kernels. This is an important factor, for larger samples in which the diffusion paths for water vapor might be larger would be expected to take longer to reach equilibrium. Data obtained are presented in Table II.

TABLE II

EFFECT OF RAPID EXCHANGE OF WATER VAPOR BETWEEN WHEAT KERNELS AND ATMOSPHERE ON WATER CONTENT DIVERSITY OF THE KERNELS [1]

Wheat variety	Treatment	Period sealed for equilibration after treatment	Mean water content	Standard deviation
			%	%
Als [2]	Original sample	—	18.33	0.19
Als	Dried 0.2 hour	0	17.79	0.19
Als	Dried 1 hour	0	16.37	0.25
Als	Dried 2.5 hours	0	15.54	0.52
	Dried 2.5 hours	73	15.35	0.22
Als	Dried 5.5 hours	0	12.67	0.92
	Dried 5.5 hours	137	12.88	0.16
Als	Damped 4.8 hours	0	20.86	0.40
	Damped 4.8 hours	92	20.55	0.22
Manitoba [3]	Original sample	—	13.88	0.27
Manitoba	Dried 6 hours	0	11.83	0.54
Manitoba	Damped 6 hours	0	19.44	0.75
	Damped 6 hours	168	19.60	0.18
Manitoba	Damped 6 hours	0	19.44	0.44
	Damped 6 hours	6	19.30	0.35
	Damped 6 hours	18	19.08	0.30
	Damped 6 hours	260	19.41	0.17

[1] Table also shows subsequent decrease in water content diversity as result of storage for varying periods in a sealed container.
[2] Als is a soft white winter wheat of Scandinavian origin.
[3] "Manitoba" wheat is a sample of the commercial grade No. 1 Manitoba wheat as exported from Canada. It probably consisted entirely of Thatcher and related hard spring wheats.

It is noticeable that the two samples of Manitoba wheat which were damped for 6 hours differ considerably in the amount of diversity which this treatment produced; in one case the standard deviation is 0.75, in the other 0.44. This failure of apparently identical treatments to produce similar amounts of diversity has been observed several times.

Distribution and Nature of the Factor Which Controls Rate of Water

Vapor Exchange. The increase in diversity due to rapid exchange of water vapor shows that the rate of water vapor exchange depends partly on a factor which is not equal for all kernels. If the values for this factor are normally distributed among the kernels, the water content frequency distribution will remain normal after a damping or drying treatment, even though the standard deviation is increased.

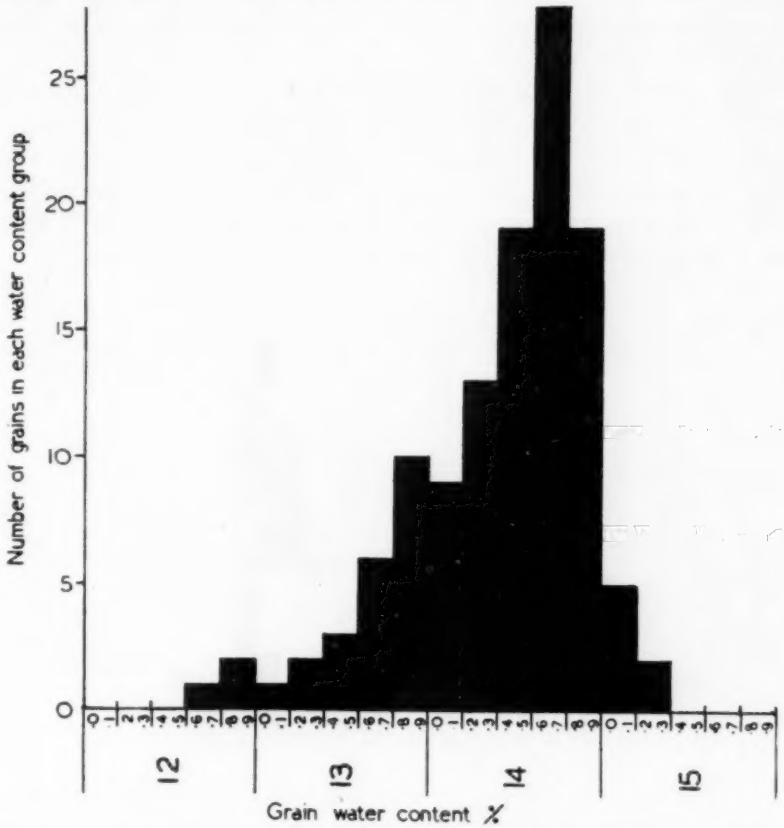

Fig. 2. Distribution of water content in 120 kernels of wheat (variety "Als") immediately after drying for 6 hours at room temperature. Initial water content was 18.3% and standard deviation 0.19; final mean water content was 14.4% and standard deviation 0.515. Skew distribution, which results from rapid drying, is well shown.

Examination of the data shows that the water content frequency distribution often becomes skew as a result of a damping or drying treatment which implies that the distribution of the factor controlling water vapor exchange is itself often skew.

Rapid drying of the soft wheat "Als" produces a skew distribution curve whose bias is towards the higher water contents. This is illustrated by the results shown graphically in Fig. 2. With initial water content 18.3% and standard deviation 0.19, 120 kernels of Als were

dried for 6 hours at room temperature in the desiccator with a fan, thereby reducing the mean water content to 14.40% and increasing the standard deviation to 0.515. The skewness of the distribution, with its bias towards the higher water contents, is fairly clear from Fig. 2. The prolonged "tail" towards low water contents indicates that there is a small proportion of kernels which lose water more rapidly than the majority. The distribution is skew because there is not a similar proportion of kernels which lose water much more slowly than the majority.

This is the only set of data available for a large number of kernels. The remaining relevant data (some of which are shown in Fig. 3) consist of 40-kernel sets which are barely large enough to show this effect. Fig. 3A shows a further set of Als, dried for 5½ hours, in which the skew distribution is apparent. Fig. 3B shows the effect of 6 days' storage in an airtight container of a portion of the same sample.

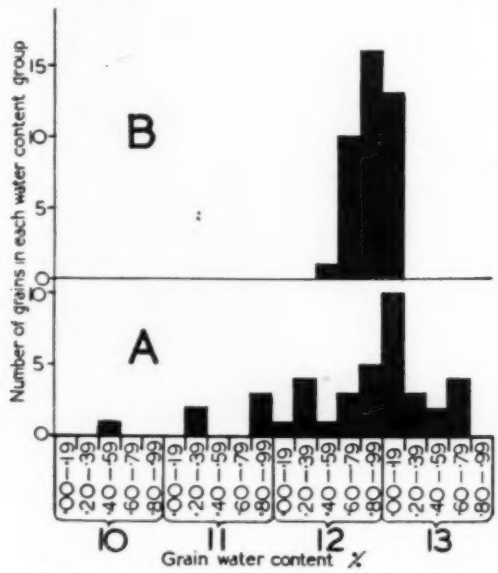

Fig. 3. Distribution of water content in 40 kernels of wheat (variety "Als"): (A) immediately after drying for 5½ hours at room temperature; (B) after 6 days' storage in a small sealed container immediately subsequent to drying.

Investigation on these lines has not been extended further since it is apparent that the present technique is not well adapted for the purpose. Further investigations would be more fruitful if based on a study of the rates of water exchange of individual kernels.

Distribution of Water Content in Mixed Wheats. Samples were taken in a small country mill [4] where English wheats from various

[4] Coxes Lock Mill, Weybridge. The author is indebted to Sebert Humphries for permission to take the samples.

sources were at the time being mixed with Manitoba and Dark Hard Winter wheats to form the grist. The objects in taking these samples were: (1) To ascertain what range of water content actually existed in the grist entering the first break rolls. (2) To study the equilibration of the sample when sealed at room temperature in order to see whether widely different wheats would settle to considerably different water contents.

At the time of sampling, English wheat was being drawn from a silo bin which contained several lots from different sources. These lots formed consecutive layers in the bin (and presumably exchanged water very slowly) but became mixed during withdrawal of the grain from the bottom of the bin. On emerging from the bin, the wheat was briefly washed, whizzed, and dried. A second sample was taken immediately after this treatment. Mixed Manitoba and Dark Hard Winter wheats (which were not sampled) were not washed in any way but were mixed with the English wheat soon after the latter left the drier. The mixture was made in the proportion 65% Manitoba and Dark Hard Winter to 35% English. The third sample was of this mixed grist, which had probably lain in the grinding bins for only a few minutes, and was taken at the point of entry to the first break rolls.

Each sample consisted of 40 kernels, which were immediately isolated in separate glass tubes, and a 30 ml. mass sample which was tightly sealed. Lots of 40 kernels were withdrawn from these from time to time for study of the equilibration process.

The results of these water content determinations are shown graphically in Fig. 4. Fig. 4A (untreated English wheat) shows the exceedingly wide range of water content (standard deviation = 1.266) which results from the mixing of small lots in the intake bins during the course of drawing off. Fig. 4D shows that washing and slight drying have not reduced this range (standard deviation 1.534). Fig. 4G shows the result of mixing 35% of the very wide range English wheat with double the quantity of the (presumably) much more homogeneous Dark Hard Winter and Manitoba mixture (standard deviation 1.222). This mixture is the grist taken as it entered the first break rolls.

The successive stages of equilibration of these samples in 30 ml. lots at room temperature are shown in Fig. 4B and 4C, 4E and 4F, and 4H, J, and K. Two facts emerge from a study of these: (1) Equilibration is very slow—even at room temperature (15°–25°C.) and in a very small container whose longest dimension was 10 cm. (2) When equilibration is presumably nearly complete the range of variation remains quite wide, a fact which reflects the varied origin of the wheats.

This is most evident in Fig. 4K where the mixed grist after 51 days shows a wide and skew distribution; presumably the skewness is due to the unequal mixture of the hard and soft wheats which appear to retain low and high water-holding capacities respectively.

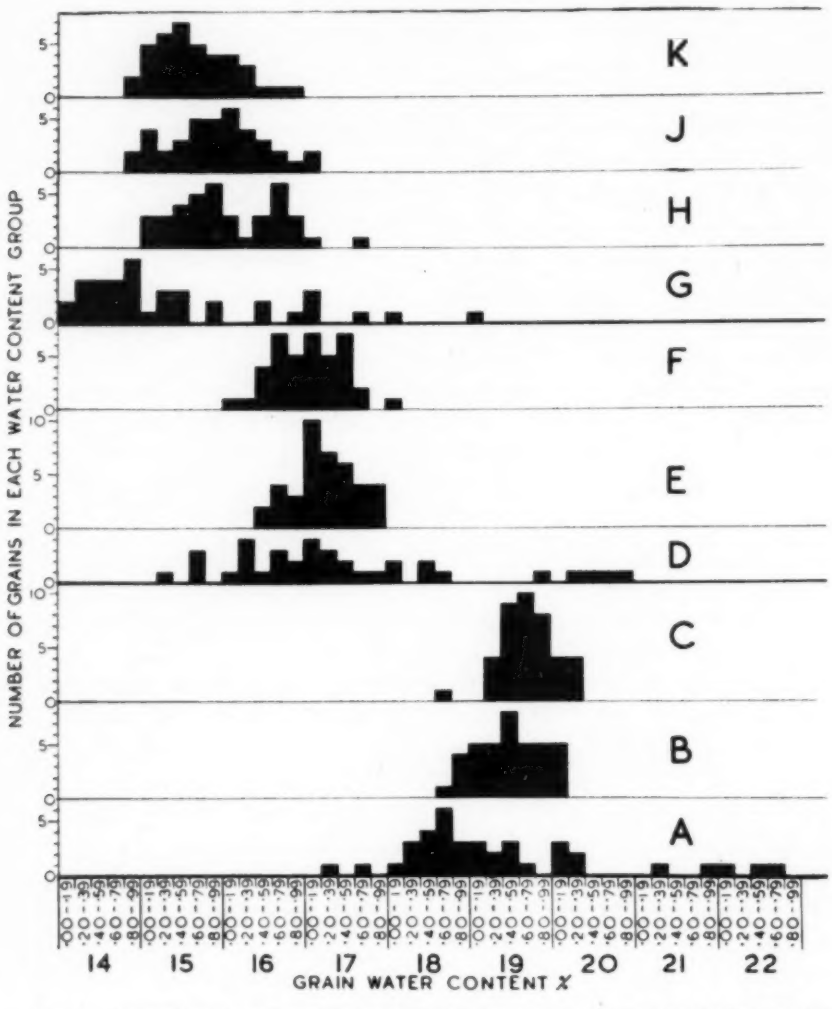

Fig. 4. Distribution of water content in mixed wheats at a country flour mill: (A) English wheat as drawn from the intake bins which had been filled with wheat from various sources. (B and C) Subsequent equilibration of the sample after 19 and 32 days respectively. (D) Mixed English wheat from the intake bins after washing, whizzing, and partial drying. (E and F) Subsequent equilibration of the sample after 19 and 32 days respectively. (G) Mixed grist (65% "Manitoba" and Dark Hard Winter wheats and 35% English) entering the first break rolls. (H, J, and K) Subsequent equilibration of the sample after 19, 32, and 51 days respectively.

Water Content Variations within a Single Ear. In July and August, 1944, a single ear was picked at random from a field of wheat (variety Squarehead Masters) on each of five occasions during the ripening

TABLE III

Water Content of Kernels of Ears of Wheat Picked at Random on Five Dates from a Single Field[1]

Kernel No.	Water content				
	1st ear July 20	2nd ear July 24	3rd ear August 1	4th ear August 3	5th ear August 8
	%	%	%	%	%
1[2]	19.5	21.2	19.3	17.1	15.2
2	19.0	36.1	20.9	17.4	14.7
3	16.5	35.9	19.0	17.5	16.7
4	21.4	20.1	18.0	17.1	15.7
5	18.1	26.7	21.2	17.1	14.6
6	21.1	34.4	21.4	17.0	15.8
7	24.5	19.7	19.6	16.3	16.6
8	15.5	36.7	18.2	17.2	15.3
9	28.8	36.2	20.0	17.2	15.3
10	32.7	34.1	20.3	16.9	15.9
11	30.4	30.7	19.7	16.7	15.0
12	33.3	48.6	22.2	16.6	15.5
13	31.5	30.3	19.0	15.8	14.5
14	33.2	35.7	22.2	15.7	14.3
15	33.4	38.9	20.9	16.3	14.1
16	26.8	35.8	20.1	16.7	15.1
17	35.2	40.9	21.7	17.3	14.8
18	23.5	35.7	22.6	16.6	15.8
19	36.8	38.4	19.3	17.4	14.4
20	36.8	38.1	21.1	16.8	15.0
21	32.6	23.3	23.5	16.5	15.7
22	39.1	38.1	23.0	17.6	14.1
23	37.3	33.0	19.0	17.3	14.1
24	25.1	38.1	21.8	17.9	14.3
25	39.7	21.4	21.0	17.7	14.9
26	32.9	34.2	23.5	18.3	15.4
27	25.9	43.7	23.7	16.8	15.2
28	40.8	34.8	22.3	17.6	15.0
29	23.9	42.3	24.0	18.6	15.7
30	40.1	36.0	21.2	18.0	15.0
31	32.8	42.4	23.6	18.0	14.0
32	39.1	41.5	20.1	17.7	14.9
33	39.9	42.8	22.6	17.6	14.9
34	41.5	39.7	22.7	19.0	15.1
35	31.9	42.8	27.0	17.9	16.1
36	41.1	43.1	23.7	19.1	16.2
37	37.1	40.8	23.5	18.8	14.8
38	—	40.4	24.9	—	14.5
39	—	42.5	21.4	—	15.2
40	—	42.8	20.4	—	17.1
Mean	30.78	35.95	21.49	17.33	15.16
Variance	60.62	49.93	3.86	0.651	0.548
Standard deviation	7.79	7.07	1.97	0.807	0.740

[1] Variety was Squarehead Master.
[2] No. 1 equals apical kernel.

period. Kernels were removed from the ears one at a time, starting from the apex, and the water contents of these (or of the first 40 kernels if the ear contained more than 40) were determined. The results of these determinations are given in Table III.

The weather was generally dull and cloudy during the period concerned, but rain and night dew were slight and infrequent. Humidities were high for the time of year but fell during most of the period between picking of the fourth and fifth ears to a normal dry summer level.

From these data the following conclusions may be drawn: (1) For this variety of wheat, in the climate of the Thames Valley in July and August, the apex of the ear tends to dry out first, though a few lower kernels may be as far advanced in drying as the apical kernels. (2) The range of variation within a single ear is very great indeed before drying out is complete. The range decreases as drying out progresses but is still much greater than is found in wheat of a single crop after storage for a few months. Presumably the range observed within a single ear represents an unsteady state, i.e., it is due to some kernels being not yet in equilibrium with the atmosphere even though the ear taken on August 8 was judged dead ripe.

The Effect of Variation in Interkernel Water Content on Desirable Size of Sample for Water Content Determination. The fact that all kernels in a sample taken for determination of water content do not have the same water content introduces an error into the determination. If the sample contains "n" kernels and the standard deviation of interkernel water content variation is σ, the standard error introduced by this source of variation will be $\frac{\sigma}{\sqrt{n}}$.

It is usual to expect that the result of a regular water content determination shall be accurate to the nearest 0.1%. If the probability of being outside this limit is to be not greater than one in one thousand times, the standard error must be not greater than $\frac{0.1}{3.29} = 0.0304$. The effect of interkernel variation on the desirable minimum sample size to insure a standard error not greater than this is given in Table IV. In the third column is given the size of the minimum sample in grams, assuming a mean kernel weight of 40 mg.

Thus a sample of 4 g. is sufficient to avoid error from this source if the grain sample is of homogeneous origin and has been stored for a few months (standard deviation up to 0.3). If the grain is of heterogeneous origin, from several fields or farms, but is well mixed and has been stored for a month or two (standard deviation up to 0.6) a sample up to 16 g. may be required. If the origin is heterogeneous and

TABLE IV

MINIMUM NUMBER OF KERNELS TO KEEP STANDARD ERROR OF MEAN BELOW 0.0304%

Standard deviation	Minimum number of kernels in sample	Weight of sample, assuming mean kernel weight of 40 mg.
		g.
0.2	44	1.7
0.3	98	3.9
0.4	174	7.0
0.5	272	10.9
0.6	392	15.7
0.7	533	21.3
0.8	696	27.8
0.9	881	35.3
1.0	1087	43.5
1.5	2440	97.6

mixing has occurred only a few hours before sampling, the sample may need to be as large as 50 g. and in extreme cases up to 100 g. may be required.

Acknowledgment

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A MODIFIED AMYLOGRAPH METHOD FOR THE RAPID DETERMINATION OF FLOUR AMYLASE ACTIVITY [1]

L. F. MARNETT, R. W. SELMAN, and R. J. SUMNER [2]

ABSTRACT

A modified method is described wherein the amylograph is utilized for the rapid determination of amylase activity by measurement of flour paste viscosity. The procedure consists essentially of employing a starting temperature of 45°C. and a temperature rise of 3.5° to 4°C. per minute, which enables a single determination to be completed in 12 minutes or less. The method is highly correlated with the standard amylograph method now in use. General principles are discussed for the possible design of a simple, inexpensive, and rugged instrument to suit the requirements of the method described.

As the evaluation of amylase activity of bakery flours by physical measurements of paste viscosity becomes more extensively used, it has become increasingly apparent that, for application to routine control, the instruments now available for such measurements are too fragile,

[1] Manuscript received October 7, 1947; presented at the Annual Meeting, May, 1947. Contribution from C. J. Patterson Company, Kansas City, Missouri.
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too time-consuming, and too expensive. These instruments, which are described by Anker and Geddes (1), Brown and Harrel (3), and Bechtel (2), are designed primarily as research tools and as such are constructed to provide a versatility of application which is not necessary in specific routine control work.

A series of investigations was initiated to determine whether or not a more rapid procedure for determining peak viscosity with the amylograph would give results which are correlated with the present standard method.

Experimental

The conventional methods of measurement of the effect of malt upon the physical properties of flour pastes involve (1) a period of incubation during which the temperature of the suspension is increased from about 25°C. to the gelatinization range, (2) a period of gelatinization during which the temperature continues to increase. The maximum paste consistency obtained during this gelatinization is used as a criterion of the amylase activity of the flour.

To evaluate the relative importance of the various steps customarily employed in the determination of amylase activity of flour by this physical method, the effect of various incubation periods over a series of temperature ranges was studied by means of the amylograph. The results of an experiment in which two flours were submitted to amylograph analysis by the method of Selman and Sumner (4), and were then submitted to a procedure whereby the suspensions were made up at 45°C. and were gelatinized in the amylograph, the thermoregulator of which was started at 45°C., are shown below:

	Paste viscosity—(amylograph units)	
	Starting temperature	*Starting temperature*
Sample number	*25°C.*	*45°C.*
1	940	830
2	480	420

The results showed that a less viscous paste is formed by the latter procedure. This means that either more enzymic breakdown of the starch takes place when a large part of the incubation period is eliminated, or else that the incubation period provides an effect which increases the potential paste viscosity of the flour suspension. If there is thermal inactivation of the enzyme system during the incubation period, the former supposition may be true, although the indicated difference in enzyme activity is about 10%. The above findings were observed when using flour suspensions of 22.2% concentration and are

contrary to those of Anker and Geddes (1) who, when using 9.1% wheat starch suspensions, found that maximum paste viscosity increased with increases in starting temperatures exceeding 25°C.

The results would appear to indicate that there is virtually no enzyme action affecting the gelatinization characteristics of the suspension until the starch has started to gelatinize. The greater part of the starch conditioning effect must then take place between the temperature of the beginning of gelatinization and the temperature at which maximum viscosity is reached. It then becomes apparent that the only requirements which must be met in order to apply the method of measurement of paste characteristics to routine malt control are (1) that the suspension be heated from the gelatinization point to the point of maximum consistency at a reproducible rate which will vary inversely with the sensitivity desired and (2) that the consistency of the paste be continuously measured during this process by a system or device which will permanently indicate the maximum consistency reached.

To obtain some background information on the potentialities of these findings with respect to the speed of determination and its relationship to sensitivity, the amylograph was used with the following procedure:

Eighty-five grams of flour (weighed on a 14% moisture basis) were suspended in 455 ml. of dilute citrate-phosphate buffer (ph 5.3) [3] at a temperature of 45°C. The suspension was placed in an amylograph the bowl of which was already warm. The amylograph thermoregulator, which had been previously set at 45°C., was manipulated manually at twice the normal rate of drive. This operation was continued until the flour suspension had reached its maximum gel consistency as indicated by the amylograph recording device. Ten to 12 minutes were required to reach this point. With this particular instrument, such a procedure produced a temperature increase of 3.5° to 4°C. per minute, depending upon the line voltage at the time of determination, since the heating system of the instrument was taxed to maximum capacity by this rate of temperature increase.

The reproducibility of maximum consistency obtained by this procedure was excellent if the line voltage was constant. The degree of variation of line voltage encountered in one day was sufficient to introduce an error of plus or minus 30 units, including the manipulation error of the instrument, since the enzyme effect, of course, varied inversely with the rate of temperature increase, whereas the rate of temperature increase varied approximately directly with the square of the voltage.

[3] The composition of this buffer is citric acid 0.105% and dibasic sodium phosphate 0.148%.

In evaluating the practicability of the principles involved, this variation of enzyme activity with line voltage is not an inherent source of error, since any instrument specifically designed for this purpose would require a heating capacity adequate to provide a surplus over that required to produce the desired rate of temperature increase.

When increments of malted barley flour were added to several samples of unmalted flour, and the samples submitted to the above analytical procedure, the maximum consistency obtained varied inversely with the quantity of malt added, according to the relationship demonstrated in Fig. 1. The variation of apparent paste viscosity with malt concentration is sensitive enough for control purposes.

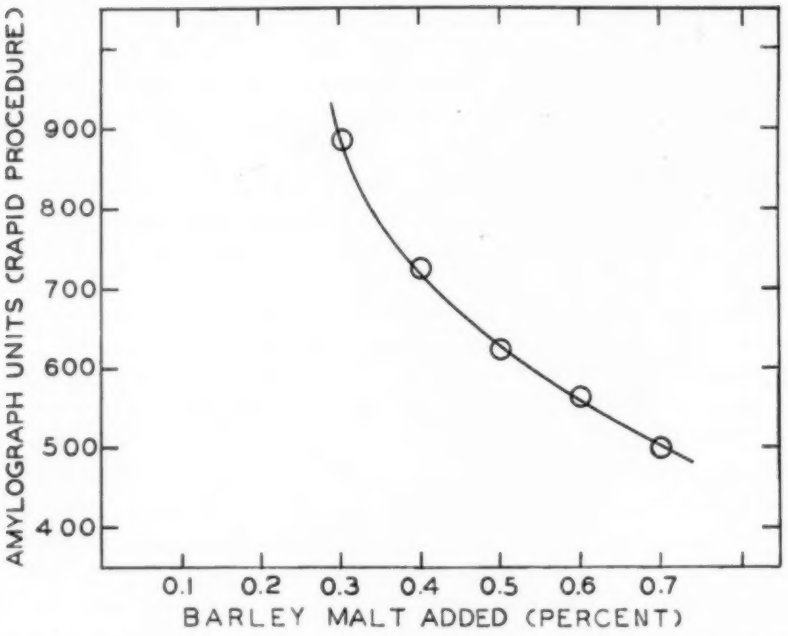

Fig. 1. Relation between maximum consistency of wheat flour pastes and quantity of barley malt added.

To check the correlation of the experimental procedure with amylograph values obtained by the standard procedure, 36 flour samples of varied types covering a wide range of alpha-amylase activity were analyzed by both procedures. The experimental values were plotted against the conventional amylograph values obtained by the procedure described by Selman and Sumner (4). The results are shown in Fig. 2. A straight line relationship exists between the two methods, and a positive correlation coefficient of 0.99 is shown. No attempt was made to hold the line voltage constant while obtaining these data. The variation was from 112 to 118 volts.

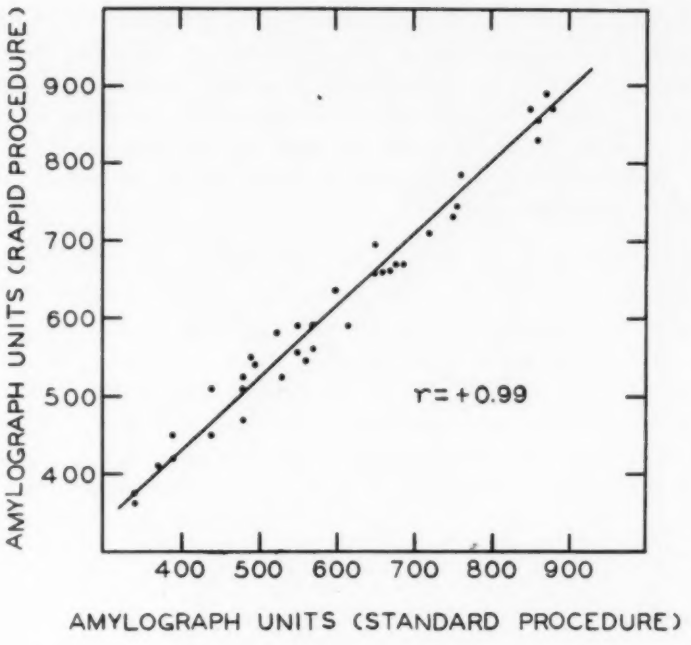

Fig. 2. Relation between maximum consistencies of flour pastes determined by the rapid and standard procedures.

Discussion

The initial temperature and the rate of temperature increase employed in this work were chosen arbitrarily, the object of the work being to determine whether malt activity could be satisfactorily evaluated by a method measuring only that effect of amylase which takes place after gelatinization has started. It is quite possible that the determination could be made at an initial temperature as high as 55°C., which would cut several minutes from the running time.

The rate of temperature increase is the factor which limits the sensitivity of the method, since a more rapid rate of heating would decrease the period of exposure of the gelatinized starch to the enzyme action, which would, in turn, decrease the consistency differential between the paste viscosity produced by a flour of low amylase content and that produced by a flour of high amylase content.

In routine mill diastatic control, this rate of temperature increase could be adjusted to suit the demands of the individual mill, since extreme sensitivity is of no advantage if the malt feeding equipment cannot accurately measure malt within narrow limits. It is obvious that by using the highest possible initial temperature, and by using the maximum rate of heating which will provide the desired degree

of sensitivity, a method of malt evaluation can be devised which will permit very rapid spot checks of the malt content of flour. Such a determination might be carried out in a sufficiently short period of time as to permit constant diastatic control of the flour in process, instead of providing a check on the flour already packaged.

To apply such a method, an instrument is needed which will meet the following requirements:

(1) It must indicate the maximum paste viscosity reached during the determination, but it is not necessary that a kymograph be used to record the entire course of consistency change.

(2) It must provide a reproducible rate of temperature increase, although the rate of temperature increment need not be constant, so long as this selected rate of temperature increase is reproducible from one determination to another. That is, immersion in a jacket or bath at constant temperature might be used instead of a system of uniform thermoregulation, if the jacket or bath were maintained at a temperature well above the gelatinization point of the flour suspension. A temperature of 95°C. or above is considered appropriate.

(3) It must be easily cleaned and durable in design.

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THE ACTION OF BETA-AMYLASE ON CORN AMYLOSE[1]

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ABSTRACT

Corn amylose, having a degree of polymerization of 455 and an iodine binding power of 19.2%, was prepared in 20.8% yield from defatted corn starch by the butanol precipitation method of Schoch. After dissolving in alkali and adjustment of the pH, the hydrolysis of the amylose solution by beta-amylase was studied by removal of portions of the reaction mixture, analyzing for reducing sugars, and recovering the unconverted high polymeric material by alcoholic precipitation. Analytical results showed that, at least during the first half of the hydrolysis, substantially only unconverted amylose and sugars with a reducing value equal to maltose were present. Inspection of unconverted amylose residues showed, except in the final stages of the hydrolysis, an unexpectedly small decrease in viscosity and degree of polymerization values, a decrease in reducing value, and an increase in iodine complexing power, compared to the original amylose. These data show that when a hydrolysis of amylose by a limited addition of beta-amylase is interrupted, only sugar and unconverted residues which have very nearly the same average size as the original sample are obtained. These results may be interpreted by assuming that when the enzyme makes contact with an amylose molecule, this chain is hydrolyzed to maltose before the enzyme attacks another chain.

The action of beta-amylase on starch is known to be a successive splitting of maltose units, by hydrolysis, from linear structures. These may be either unbranched, alpha-glucopyranoside molecules or unbranched portions of more complexly constituted molecules. The end product in the case of unbranched amylose chains is maltose, and yields approximating 100% of theory have been reported (10, 7).

The purpose of this paper is to extend our knowledge of the mechanism of beta-amylase action.

Materials and Methods

Preparation of Corn A-Fraction (Corn Amylose). The linear polymer fraction of defatted corn starch was prepared according to the method of Schoch (13) using Pentasol in the primary separation and butanol to recrystallize the product from aqueous solution. The yield was 20.8%. Iodine absorption by the product, measured potentiometrically by a modification (8) of the method of Bates, French, and Rundle (2), was found to be 19.2%.

Preparation of Beta-Amylase. The enzyme was prepared from barley according to the procedure outlined by Kerr and Trubell (4) and further purified according to the method of Ohlsson (12). Free-

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dom from alpha-amylase was determined by using amylopectin, limit dextrin as substrate and noting the absence of new reducing groups formed, as measured by ferricyanide oxidation.

Enzyme Hydrolysis. Fifty grams of the corn amylose were dissolved at room temperature in 250 ml. of 2 *N* potassium hydroxide solution. The solution was diluted to 10 liters and adjusted to pH 5.8 with hydrochloric acid. This preparation was heated to 47°C., 25 ml. of a beta-amylase preparation were added, and the final volume adjusted to 12,500 ml.

Hydrolysis was allowed to proceed at 47°C. and aliquot portions were removed for testing and for the recovery of products at stated times. The per cent conversion to maltose at different time intervals was determined by reduction of ferricyanide according to the method used by Kerr, Trubell, and Severson (5).

Samples of the conversion liquors were taken at the times indicated, butanol added to the extent of 10%, by volume, the solution brought quickly to a boil, and partially cooled. Then another 10% by volume of butanol and a volume of methanol equal to the amount of water present were added. The solution was allowed to cool to room temperature by standing overnight. The precipitate was recovered by centrifuging and recrystallized by dissolving in 300 ml. of boiling water (115 ml. for sample C, Table I), saturating the solution with butanol,

TABLE I

HYDROLYSIS OF CORN AMYLOSE WITH BETA-AMYLASE

Sample taken for precipitation	Hydrolysis time	Hydrolysis to maltose	Monomolecular reaction rate constant	Unconverted, high polymer fraction		Characteristics of unconverted high polymer			
				Calculated	Found	Ferricyanide No.	Viscosity in ethylenediamine	Molecular extinction coefficient	Degree of polymerization
	min.	%	k_1 (*min.*$^{-1}$)	*g.*	*g.*		$[\eta]$	$\times 10^{-3}$	DP_n
	0	0	0.0044	(50)	(50)	1.43	1.258	3.73	455
	30	10.3	0.0042	—	—	—	—	—	—
	60	19.7	0.0040	—	—	—	—	—	—
A	90	28.0	0.0035	6.6	6.2	1.33	1.162	4.13	330
	180	43.0	0.0030	—	—	—	—	—	—
B	260	52.6	0.0028	6.5	6.2	1.27	1.193	4.11	365
C	1260	90.7	0.0006	2.1	0.9	6.30	0.240	3.06	120

and allowing the mixture to cool slowly overnight to room temperature. The recrystallized products were washed twice with 100-ml. portions of cold water saturated with butanol and then with four, 400-ml. portions of absolute methanol. The methanol was removed by plac-

ing the crystals in a vacuum desiccator over sulfuric acid at room temperature.

Measurement of Viscosity. The intrinsic viscosity of the various amylose products was determined in ethylenediamine using substantially the procedure described by Kerr (8).

Reducing Value. The ferricyanide reducing value of the products was determined in a manner analogous to the determination of percentage conversion of amylose. However, the results are expressed as milliliters of tenth normal sodium thiosulfate, equivalent to 1 g. of amylose product.

Molecular Extinction Coefficients. The molecular extinction coefficients of the iodine complexes were determined using a Coleman, Model 10S spectrophotometer. Exactly 0.1000 g. of amylose product was dissolved in 3.33 ml. of 2 *N* potassium hydroxide and transferred to a 2-liter flask with 1.5 liters of water. The pH was adjusted to 5.0 with a solution of hydrochloric acid and 20 ml. of a standard solution of iodine in potassium iodide was added. The latter contained 0.5 g. of iodine and 0.75 g. of potassium iodide per 100 ml. The volume of the mixed solution was made up to 2 liters. This solution was further diluted fivefold with water for spectrophotometric measurements over the wave band range of 550 to 660 mμ. The per cent light transmission at the wave length which gave the minimum value was selected in each case for calculation of the molecular coefficient of extinction according to the equation:

$$k = -\frac{\log T}{dc}$$

wherein T is the fractional transmission
d is the cell depth of 1.3 cm
c is 0.047875 molar iodine concentration.

Acetylation. Acetates of the amylose products were prepared by a modification of the method of Mullen and Pacsu (11). The modification consisted of adding fused sodium acetate to the reaction mixture after distilling water from the aqueous pyridine solution and a primary reaction with acetic anhydride.

The reaction mixture was refluxed for two additional periods of one hour each after addition of the sodium acetate. By this modified procedure yields of acetates were obtained in excess of 90% of theory and which had acetyl contents between 44.0 and 44.8%.

Osmotic Pressure. Osmotic pressure measurements were made on the acetates of the amylose products in chloroform using a cell substantially as described by Fuoss and Mead (3). The cell was enclosed

in a glass-windowed cabinet which was maintained at 30°C. ± 0.05 degree. Readings of liquid levels were made by the use of a Gaertner, model M908, cathetometer with an accuracy of ±0.05 mm. The dynamic method of measurement described by Fuoss and Mead was used. The calculated values were checked frequently with static values.

Membranes consisted of uncoated No. 450 cellophane which were treated by soaking successively in water (50°C.); 50% water—50% ethanol; 95% ethanol; 50% ethanol—50% amyl alcohol; 100% amyl alcohol; 75% amyl alcohol—25% chloroform; 50% amyl alcohol—50% chloroform; 25 per cent amyl alcohol—75% chloroform; and chloroform. Each soaking period consisted of from 6 to 24 hours.

The molecular weights, expressed as degree of polymerization (number average of glucose units), were calculated from the osmotic pressure of the acetates at zero concentration (by extrapolation) as follows:

$$DP_n = \frac{RT}{[\pi/c]_{c=0} \times m} = \frac{2.57 \times 10^5}{[\pi/c]_{c=0} \times 288}$$

where R is the gas constant

T is the absolute temperature
π is the osmotic pressure expressed as cm head of water
c is the concentration of the acetate in grams per 100 ml of chloroform
m is the molar weight of an anhydro, glucose triacetate group.

Results and Discussion

The results of the hydrolysis of corn A-fraction (amylose) by beta-amylase and of various tests performed on three intermediate samples recovered during the course of the hydrolysis are shown in Table I. The reaction rate of the hydrolysis does not correspond to a zero order reaction since equal weights of maltose are not formed in each successive unit of time. Neither does the monomolecular reaction equation, wherein the rate constant is a function of the amount of unconverted amylose, give constant values for K_1 although constant values have previously been reported (7) when the shorter amylose chains are hydrolyzed in dilute solution. For the whole amylose fraction of corn starch the rate constants decrease progressively as the hydrolysis continues. By extrapolation of these K_1 values to zero time, an initial K_1 value of 4.2×10^{-3} was estimated. This is less than half the value of 9.8×10^{-3}, found for the hydrolysis of an equal weight concentration of the shorter chain lengths such as are obtained by aqueous leaching of corn starch (6). The greater rate for shorter chains may be ex-

plained, in part at least, by the fact that there are a greater number of reactive chain ends, per unit weight, of the shorter than of the longer chain length sample.

The amounts of high polymeric material recovered from the conversion of corn A-fraction agree fairly well with calculations based on the assumption that at any time during the hydrolysis (at least up to 50% conversion) the only substances present are high polymers and sugars with a reducing value substantially equal to that of maltose.

The nature of the unconverted polymers is indicated by measurements of viscosity, reducing value, molecular extinction coefficient of the iodine complexes and osmotic pressure studies. Up to a point corresponding to 50% conversion of the amylose to maltose, the intrinsic viscosity of the unconverted material decreases very little. This result might be anticipated from the following considerations. The shortest amylose chains in the original sample would be expected to contribute little to its viscosity, since viscosity is a function of weight average molecular weight (9). It would also be expected from rate studies that a greater number of these shortest chains would be hydrolyzed per unit time than the longer, leaving an increasing proportion of longer chains as the hydrolysis time was extended.

However, if the reaction proceeded uniformly so that all molecules were progressively hydrolyzed, one would also expect that by the time half the weight of the amylose sample was converted, the longest chains would be materially reduced in chain length also. This is apparently not the case, particularly as in the present instance, when a limited amount of enzyme was used. Osmotic pressure measurements confirm this conclusion. Calculations of degree of polymerization from the osmotic pressures of the acetates in chloroform give a value of 455 glucopyranose units for the whole corn amylose sample and 365 units for the amylose which remains after 52.6% conversion to maltose. An examination of the reducing value of the amylose chains, which were recovered from the earlier stages of the hydrolysis, shows that there are very nearly the same number of reducing end groups per unit weight as in the original amylose. This is also fair evidence that the number average molecular weight of the recovered amylose is of the same order as that of the original material. Therefore it seems improbable that the action of beta-amylase is a gradual shortening of all chain lengths present because, if, at 50% conversion, the number of amylose chain residues were the same as the number of original amylose molecules, then the number average molecular weight at this point would be very nearly half of the original molecular weight.

Alternately, one may assume that the longer chains are preferentially hydrolyzed and that the range and distribution of molecular

sizes in the original amylose are such that when 50% conversion to maltose has been attained, the average chain length of the original sample is only slightly reduced as the result of the complete hydrolysis of a relatively few high molecular weight chains. However, if we assume that there are only unbranched chains in the amylose, then, in view of the work of Baldwin, Bear, and Rundle (1), our values for molecular extinction coefficients indicate that in the early stages of conversion the longer molecules of high iodine binding power have been preserved and that the sugar produced has been from the hydrolysis of the shorter chains of low iodine binding power. The value of 4.11×10^3 after 52.6% hydrolysis is significantly higher than 3.73×10^3, the value for the original corn A-fraction.

The increased iodine staining power of the unconverted residue is not consistent with the assumption that its high average molecular weight is due to an accumulation of amylopectin impurities as the hydrolysis of linear chains progresses.

On the basis of present concepts the data given can be interpreted by concluding that once an active group on the enzyme makes contact with a starch chain, hydrolysis goes to completion before another starch chain is attached. At any time during the hydrolysis of linear chains the product is substantially all maltose; only a very small amount of intermediate products is present. Shorter chains are hydrolyzed at a greater rate, per unit weight, than longer and it seems probable also that the shorter chains are more readily contacted by the enzyme.[2]

The above conclusion is important when it is desired to modify the linear polymer characteristics of whole starch by amylase action. When beta-amylase is used for this purpose, or enzymes which contain this component, the linear polymer is modified in direct proportion to the production of sugar and in the modified starch product the proportionate weight of the linear component is eventually lost for applications where high polymeric materials are required.

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[2] From the conclusion given for the mechanism of beta-amylase action it follows that by varying the ratio of the number of active enzyme groups to the number of amylose molecules present, one may obtain from the hydrolysis unconverted residues which have molecular weights less than, equal to, or even greater than the average for the original amylose. For example, if a large excess of enzyme were added so that there were more than one active enzyme group for each starch molecule, then one might expect that all amylose chains would be hydrolyzed simultaneously. On the other hand if only a very limited number of active enzyme groups were present, it would appear that only a very few of the short chain lengths would be hydrolyzed.

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ADSORPTION MEASUREMENTS ON FLOUR USING RADIOACTIVE ISOTOPES [1]

J. W. T. Spinks [2] and C. I. Tollefson [3]

ABSTRACT

A convenient and rapid radioactive tracer method of determining adsorption of ruthenium ions by flour is described. The adsorption of Ru^{+++} by four wheat flour mill streams increased with decreasing grade of flour, and it is possible that the method may be of value as a quick method for estimating baking quality on a small sample of flour.

During the last few years a number of studies have been made on the adsorption of different materials by flour (2). The amounts adsorbed are sometimes quite small, of the order of a few gammas per gram flour. While such amounts can ordinarily be measured without difficulty using a colorimetric procedure, the determination in the presence of flour is quite tedious. In contrast to this, the determination with the help of radioactive tracers is very simple and rapid. Furthermore, the extreme sensitivity of the radioactive tracer method enables one to work on a micro or semimicro scale, a fact which could be of considerable importance when making measurements, for example, on flour from a new variety of wheat. Since radioactive iso-

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topes of practically all elements are now available in quantity, the radioactive tracer method is of general applicability and it was thought that a description of some experiments on the adsorption of ruthenium chloride by flour might be of some interest.

In any tracer experiment, a number of general factors are important. Among these are: (a) half life, (b) energy of the emitted radiations, (c) presence of interfering daughters, (d) self absorption, (e) counter standardization, and (f) statistical errors (3). In the present experiments, ruthenium (Ru^{106}), half life one year, was used. This half life is sufficiently long that no correction for the decay of Ru^{106} need be made in experiments completed within a day or so. Ru^{106} decays with emission of beta particles, energy 0.03 Mev, to give rhodium, half life 30 seconds. Rh^{106} decays with emission of beta particles, energy 3.9 Mev, to give stable palladium (Pd^{106}). The beta particles from Ru^{106} are not energetic enough to penetrate the window of the usual Geiger Mueller counter. However, any specimen of Ru^{106} which has been standing for a few minutes will be in equilibrium with its Rh^{106} daughter, whose beta particles are easily measurable. The radiations of the Rh^{106} daughter can, in fact, be used as a quantitative measure of the amount of Ru^{106} present.

Materials and Methods

A sample of radioactive ruthenium was separated from possible impurities by a perchloric acid distillation (4). Fuming perchloric acid oxidizes ruthenium to the volatile tetroxide, RuO_4, which distils off and is trapped in caustic soda solution. The octavalent ruthenium is readily reduced by alcohol to the trivalent state and the oxide, Ru_2O_3 (hydrated), precipitated. This may be centrifuged off, washed, and dissolved in a little hydrochloric acid. Half life and half thickness values indicated that the sample so purified was the Ru^{106} isotope. All samples were counted using a Geiger Mueller counter and a scale of 128 counting unit (7).

When determining the half life, the ruthenium sample was compared with a uranium oxide standard over a period of 9 months. The activity of the ruthenium was calculated on the basis that the activity of the uranium oxide standard remained constant over the period studied. This eliminated errors due to variations in counter performance. In all cases enough counts were recorded that the probable error should be less than $\pm 1\%$. All counts were corrected for background. The data for the half life of this ruthenium sample are plotted in Fig. 1. By extrapolation of the half life curve, it appears that the half life of the sample is about 360 days. The published value for the half life of Ru^{106} is one year (6).

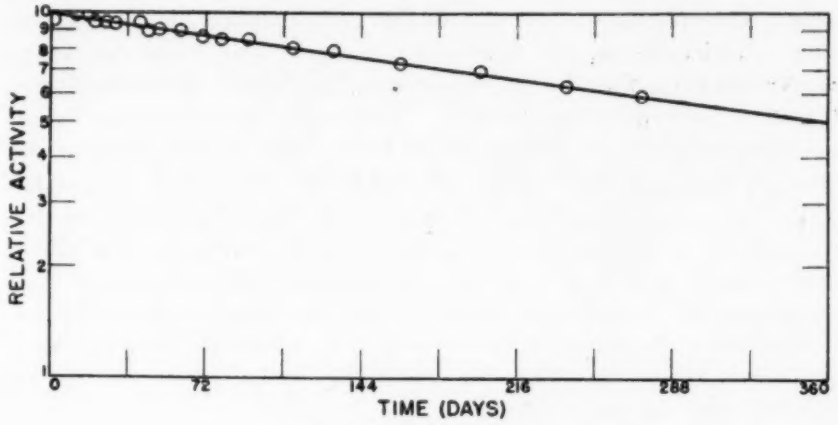

Fig. 1. Half life of Ru^{106}.

In determining the half thickness, the net counting rate is determined with and without aluminum absorber screens interposed between the sample and the counter tube. The per cent transmission through a given screen is plotted against the absorber thickness expressed in mg. per sq. cm. From this curve, the half thickness value can be determined—i.e., the thickness of aluminum required to reduce the activity of the ruthenium sample to one-half its initial value. The data for this determination are plotted in Fig. 2.

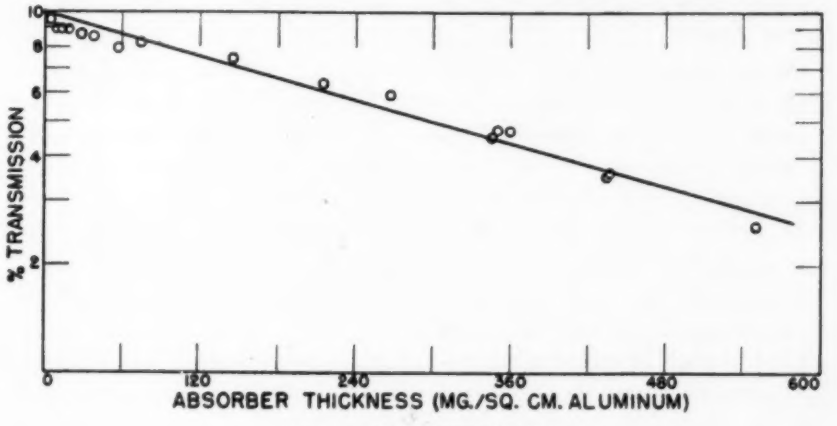

Fig. 2. Half thickness of Ru^{106}.

The half thickness of Ru^{106} as determined from Fig. 2 is 300 mg. per sq. cm. Al, and indicates that absorption of beta particles by the material itself (so called self-absorption) can be neglected.

During the course of the experimental work all radioactive residues were saved and were worked up on two occasions to recover the ruthe-

nium. The residues were combined and evaporated to dryness. Since considerable organic matter was present, and it was desired to remove the ruthenium by perchloric acid distillation, it was necessary to destroy the organic matter first. This was accomplished by means of a peroxide fusion in an iron crucible (5). The fusion cake was dissolved in water, partly neutralized with hydrochloric acid, and boiled to destroy excess peroxides. As some iron had dissolved from the crucible during the fusion, a precipitate of ferric hydroxide formed and carried down practically all the ruthenium. The precipitate was centrifuged off, washed, and subjected to a perchloric acid distillation. In the second recovery of ruthenium, the radioactive residues were evaporated to dryness and the ruthenium recovered by distillation with sulfuric acid and sodium bromate (1).

Procedure. One quarter gram samples of flour [4] were weighed into centrifuge tubes and treated with 5 ml. of solution containing radioactive ruthenium ion. The tubes were shaken by hand to disperse the flour and then placed on a "Gyrosolver" shaker for 10 minutes. The samples were next centrifuged and aliquots of the supernatant liquid taken for counting. These aliquots were dried on small watch glasses and counted. From the difference between the activities of the original and final solutions, the amount of ruthenium ion removed from solution could be calculated. In the absence of interfering effects, this was equal to the amount of ruthenium ion adsorbed by the flour. This was then converted to gammas of ruthenium ion adsorbed per gram of flour.

To determine the correction for self absorption, the residue on each watch glass was weighed. The weight varied between 5.1 and 8.8 mg., with an average value of 6.2 mg. The area of watch glass covered was about 5 sq. cm. Hence, the thickness of precipitate was in no case greater than 2 mg. per sq. cm. This would have no appreciable effect on the measured activity of these samples as ruthenium emits quite energetic radiation (maximum energy 3.9 Mev, half thickness 300 mg. per sq. cm.). Thus, for these experiments, it was not necessary to apply corrections for self absorption.

The method of calculating the amount of ruthenium ion adsorbed is quite simple. One quarter gram samples of flour were treated with 5 ml. of ruthenium chloride solution containing, say, 25 gammas Ru^{+++}. This corresponds to 100 gammas Ru^{+++} per gram flour. After shaking the sample and centrifuging, an aliquot of 1 ml. was withdrawn from the supernatant liquid. This aliquot was dried and had an activity of 566 counts per minute. A similar aliquot of the

[4] Samples of freshly milled, unbleached, untreated flours were obtained from the Quaker Oats Company, Ltd., Saskatoon.

original solution had an activity of 813 counts per minute. Since the initial amount of ruthenium ion corresponded to 100 gammas Ru^{+++} per gram flour, the final amount of ruthenium ion in solution must correspond to $\frac{566}{813} \times 100 = 69.6$ gammas Ru^{+++} per gram flour. By difference, the amount of ruthenium ion adsorbed must have been 30.4 gammas Ru^{+++} per gram flour.

Factors Influencing Adsorption of Ruthenium

Effect of pH. In some preliminary experiments a marked effect of pH on the "adsorption" of ruthenium ion by flour was observed. Since it was thought that the effect might be due, at least in part, to precipitation of ruthenium, the effect of pH on the precipitation of Ru^{+++} was investigated.

Effect of pH on the Precipitation of Ru^{+++}. A series of ruthenium chloride solutions, with pH adjusted, were put through all the steps of the regular adsorption procedure, but with no flour added. The results are recorded in Table I.

TABLE I

EFFECT OF pH ON PRECIPITATION OF Ru^{+++} (T = 20°C.)

pH	Activity left in solution, %
2.24	100.0
2.38	95.6
2.70	92.7
2.75	95.2
2.90	94.3
3.00	90.2
3.15	100.0
3.40	40.0
4.00	23.2
4.40	14.4
5.40	20.8
8.70	4.5

From Table I it is evident that ruthenium chloride solution is not stable above pH 3.0–3.2. Apparently, hydrolysis takes place at higher pH values and the hydrated oxides precipitate (1). In any case, it is necessary to carry out all the adsorption tests at as low a pH as is practicable in order to avoid this effect. As a result, it was decided to carry out all adsorption tests at pH 3.1.

Effect of Shaking Time. To determine the effect of time of shaking, samples of a "second break" flour were shaken with a ruthenium chloride solution for varying lengths of time on the Gyrosolver, centrifuged, and the activity of the supernatant determined in the usual manner. The results (see Table II) indicate that samples should be

shaken for at least 5 to 10 minutes to ensure complete dispersion of the flour in the ruthenium chloride solution. It appears that fairly reproducible results can be obtained by this method—the probable error for this experiment was ±2%.

TABLE II

EFFECT OF TIME OF SHAKING (T = 20°C.)

Sample	Shaking time, minutes	Ru^{+++} added, gamma	Activity lost, %	Ru^{+++} adsorbed, gamma
1	0.5	25	27.6	6.9
2	0.5	25	29.6	7.4
3	5	25	39.6	9.9
4	5	25	39.2	9.8
5	10	25	41.2	10.3
6	10	25	39.6	9.9
7	15	25	39.2	9.8
8	15	25	40.0	10.0

Effect of Concentration of Ru^{+++} and Grade of Flour. In order to investigate the adsorption of ruthenium ion by flour in somewhat greater detail, six samples of each of four mill streams were treated with different concentrations of ruthenium chloride solutions. These samples were shaken on the Gyrosolver for 15 minutes, centrifuged, and the activity of the supernatant liquid determined in the usual manner. The results are recorded in Table III.

TABLE III

RESIDUAL AMOUNTS OF Ru^{+++} IN SOLUTIONS AFTER TREATMENT WITH FLOURS[1] (GAMMAS Ru^{+++} PER GRAM FLOUR)

Initial amount of Ru (gammas Ru^{+++}/ gram flour)	First middlings	Second break	Third low grade	Third tailings
18.75	10.9	8.0	7.4	7.2
37.50	25.0	21.1	17.0	14.2
75.00	53.3	50.5	45.4	36.9
150.00	114.3	115.3	111.1	104.6

[1] Ash contents: first middlings 0.36%, third low grade 0.83%, second break 0.46%, third tailings 0.62%.

When the amount of ruthenium ion adsorbed is plotted against the amount of ruthenium ion remaining in solution, typical adsorption curves are obtained.

The results in Table III also indicate that the amount of ruthenium ion adsorbed increases with decreasing flour grade. For more extensive data see Tollefson (8). This parallels the requirement of im-

prover for optimum loaf volume, and indicates the possibility of correlating flour grade with adsorption of ruthenium ion.

It is known that certain flours improve in baking quality on aging and, consequently, adsorption tests were made on a number of mill streams over a period of several months. Typical results are recorded for one flour in Table IV.

TABLE IV

RESIDUAL AMOUNTS OF RUTHENIUM IN SOLUTION AFTER TREATMENT WITH FIRST MIDDLINGS AT VARIOUS DATES (GAMMAS Ru^{+++} PER GRAM FLOUR)

Initial amount of Ru^{+++}, (gammas Ru^{+++}/gram flour)	Nov. 20/46	Nov. 30/46	Dec. 18/46	Jan. 5/47	Feb. 13/47	Mar. 22/47
18.75	10.9	11.3	10.8	11.7	11.4	10.9
37.50	25.0	24.9	23.9	24.8	24.0	24.2
75.00	53.3	54.7	53.3	54.4	53.7	53.6
150.00	114.3	116.8	114.3	115.5	114.3	116.7

During the period studied there was no appreciable change in the adsorption of ruthenium ion by the different flour streams. However, during the same period, there was no appreciable change in loaf volume so that no particular conclusions can be drawn.

Acknowledgment

The authors are grateful to the National Research Council of Canada for a grant in aid of research.

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WATER SORPTION BY CORN STARCH AS INFLUENCED BY PREPARATORY PROCEDURES AND STORAGE TIME [1]

N. N. HELLMAN and E. H. MELVIN

ABSTRACT

Measurements were made of the amount of water sorbed by 10 corn starches in equilibrium with atmospheres of different relative humidities ranging from 12 to 93%. The 10 starches differed among themselves with respect to origin, concentration of sulfur dioxide in the steep water at the time of extraction, drying, and time in storage. Despite these differences in preparation, all 10 starches showed essentially the same moisture sorption. It was concluded that data secured in studies of the moisture-sorptive properties of a single corn starch can generally be applied to normally prepared unmodified corn starches.

The amount of water which corn starch will sorb in equilibrium with an atmosphere of given moisture content has been shown to be affected by intensive drying and the various treatments used commercially to prepare modified starches (2, 3). The moisture-sorptive capacity will also vary, depending upon (a) the difference between the initial and final moisture content of the starch and (b) whether moisture is being lost or gained by the starch in the approach to equilibrium. Changes in water-sorptive capacity of the latter type are analogous to those shown by many other colloidal materials, and the phenomenon is known as hysteresis. The effects of hysteresis are reversible and are thus distinguished from the permanent changes in sorptive capacity which are induced by the first-mentioned treatments.

Permanent differences in water-sorptive properties might conceivably be produced also by variations encountered normally in the preparation of starch, such as difference in origin and condition of the corn, extraction and drying procedure, and time of storage. A survey is here reported of the moisture-sorptive properties of a collection of corn starches which differed among themselves with respect to the factors just mentioned. This survey was preliminary to a detailed study of moisture sorption isotherms of corn starch and was made to determine if data obtained in such a study would be generally applicable to unmodified corn starches.

Materials and Methods

Ten corn starch samples were selected which represent different preparative methods and periods of storage. The pertinent properties of the corn starch samples are given in Table I. Approximately 2-g.

[1] Manuscript received December 8, 1947.
Contribution from the Northern Regional Research Laboratory, Peoria, Illinois, one of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE I

PREPARATORY HISTORY OF CORN STARCH SAMPLES

Sample No.	Processor	Type	Concentration of SO_2 in steep water	Method of drying	Temp. of drying, °F.	Time of drying	Time in storage (months)
L-1	Laboratory	Prepared from Iowa hybrid 939 corn (1942 crop)	0.20%[1]	Forced draft oven	105	7 hrs.	33
L-2	Laboratory	Prepared from Iowa hybrid 939 corn (1942 crop)	0.30%	Forced draft oven	105	7 hrs.	39
L-3	Laboratory	Prepared from Iowa hybrid 939 corn (1942 crop)	0.40%	Forced draft oven	105	7 hrs.	37
L-4	Laboratory	Prepared from Iowa hybrid 939 corn (1944 crop)	Distilled water used	Forced draft oven	105	3 hrs.	13
PP-1	Pilot Plant	Prepared from Iowa hybrid 939 corn (1942 crop)	Distilled water used	Forced draft oven	110	26 hrs.	17
C-1[2]	Company A	Prepared from soft corn	Normal	Proctor Schwartz			1
C-2	Company A	Pearl	0.02–0.08	Kiln		20 hrs.	56
C-3	Company B	Powdered	Max. 0.18	Proctor Schwartz	190–250	25 min.	55
C-4	Company C	Pearl	0.04–0.20	Proctor Schwartz	190–220	1 hr.	56
C-5	Company C	Pearl	0.04–0.20	Tunnel kiln	105–240	18 hrs.	56

[1] For the laboratory preparations, the SO_2 concentration is that of the steep when started. No further SO_2 is added, and the water is not changed during the process.
[2] Scott viscosity only 15, whereas that for normal corn starch is 100 or over.

samples of the various starches at the moisture content at which they were normally stored (10–13%) were placed in aluminum moisture dishes. The samples were then placed in a vacuum desiccator over a saturated salt solution selected to give the appropriate vapor pressure of water. The solutions used are given in Table II and were selected

TABLE II

SALT SOLUTIONS USED FOR CONSTANT RELATIVE HUMIDITY

Salt	Relative humidity	Reference
KNO_3	93.5	(4)
KCl	85.0	(4)
$NaNO_3$	74.5	(4)
NaBr	56.4	(1)
K_2CO_3	43.0	(4)
$KC_2H_3O_2$	20.0	(4)
LiCl	12.2	(1)

from the *International Critical Tables* (4) and the *Physikalisch-chemische Tabellen* (1) tabulations. To hasten equilibrium, the desiccators were evacuated with a two-stage, rotary, oil, vacuum pump and, to ensure complete removal of air, were pumped for 1 hour after the pressure had been reduced to the pressure of the water in the salt solution. The desiccators were then placed in a constant-temperature room, controlled at 25 ±1°C., and allowed to stand 2 to 4 weeks, a period shown by other trials to be ample for equilibration.

At the conclusion of the period of equilibration the pressures of the atmospheres within the desiccators were measured with an ordinary closed-end, mercury, U-tube manometer to assure that the pressure of water was that anticipated from the literature data. Dry air was then admitted, the desiccator opened, and the moisture content of the starch samples determined. Moisture contents were determined by heating in a vacuum oven at 105°C. for 24 hours. Tests of the reproducibility of results by use of this technique showed that within the same desiccator the per cent moisture content of triplicate samples agreed to within 0.3. The vapor pressures of water in the desiccators probably varied slightly for a given salt solution as a result of slight under or super saturation during the course of equilibration inasmuch as the rate of salt solution or precipitation probably could not follow the rate of temperature drift. This gives rise to an uncertainty in the relative humidities designated in Table III, which was reflected in the manometer readings and in a variation in the per cent of water by as much as 0.6 encountered in the moisture content of a standard starch between duplicate desiccators employing the same salt.

TABLE III

WATER SORPTION OF CORN STARCH SAMPLES AT VARIOUS HUMIDITIES AT 25°C.

Sample	Per cent water sorbed in equilibrium with air of designated relative humidity						
	93%	85%	75%	56%	43%	20%	12%
L-1	26.5	22.1	19.4	16.7	13.9	10.6	7.7
L-2	26.1	22.1	19.5	16.7	—	10.6	7.6
L-3	26.3	22.1	19.6	16.3	14.2	10.6	—
L-4	27.0	22.3	19.7	16.3	14.2	10.6	7.6
PP-1	26.4	21.9	19.2	16.0	13.9	10.5	7.6
C-1	25.7	22.0	18.9	15.8	13.5	—	7.2
C-2	26.6	21.8	19.1	15.5	13.2	10.3	7.5
C-3	26.2	22.2	19.0	15.7	13.4	10.4	7.6
C-4	25.6	21.9	18.9	15.7	13.4	10.4	7.7
C-5	26.0	22.1	19.0	16.0	13.8	10.6	7.6
Average	26.2	22.0	19.2	16.0	13.7	10.4	7.6
PP-1-wet	26.5	22.7	20.0	—	14.2	10.3	7.4
C-1-wet	26.9	22.9	20.0	16.6	14.1	10.3	7.4

Results and Discussion

The uniformity of moisture sorption by the various corn starches is particularly striking. The amounts of water sorbed by the starches at the seven humidities employed are shown in Table III. The concentration of sulfur dioxide in the steep water is shown to have little effect on moisture sorption by the first four starches tabulated. Sample SD-144, which was extracted from soft corn, appears, with respect to its moisture-sorptive properties, to have been relatively unaffected by its origin. The starches from laboratory extractions exemplified by samples L-1, L-2, L-3, and L-4 show little difference with respect to the water-sorptive properties as compared to commercial preparations C-1, C-2, C-3, C-4, and C-5. These laboratory and commercial preparations differed from each other in time of storage as well as in methods of drying. Although rigorous drying and modification treatments have been shown to affect moisture-sorptive properties (2, 3), it appears that within limits of normal corn starch preparatory practice, the variables of origin, sulfur dioxide concentration in the steep liquor, drying practice, and time of storage are of little significance. For moisture sorption studies, a single representative corn starch can be studied with confidence as to the generality of the results as applied to other unmodified corn starches. This conclusion cannot be applied to starches from other plant sources without further investigation, since it has been shown for potato starch that a preparative treatment involving combined heat and moisture would produce changes (2).

Within the general conclusion that the samples showed uniform sorption, the possibility exists that variations in preparative history may cause minor variations in water sorption. Such variations must be so small, however, that they can be obscured by the nonpermanent changes in moisture sorption shown in hysteresis which are of the same order of magnitude as the differences in sorptive capacities shown by the various starches studied. The effect of hysteresis is shown in samples of pilot-plant corn starch (PP-1-wet) and the freshest available (C-1-wet) commercial corn starch which were wet with sufficient water to cover completely the starch before equilibrating with moist air.

The viscosities of pastes from these materials lay over a relatively wide range.[2] The uniformity of the moisture-sorptive properties and the nonuniformity of their paste viscosities clearly indicate that hydration measured in terms of moisture sorption of whole granules at room temperature is not of primary importance in controlling the paste viscosity. If one wishes to consider that paste viscosity is controlled by hydration, then it must be hypothesized that this is effected through new sites of hydration which arise in the process of gelatinization.

Acknowledgment

The authors wish to thank Dr. M. M. MacMasters of this laboratory for providing the samples used in this investigation and her helpful discussion during the progress of this work. The interest of Dr. R. T. Milner is gratefully acknowledged.

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BOOK REVIEWS

Volumetric Analysis. Volume II, Titration Methods. By I. M. Kolthoff and V. A. Stenger. 374 pp. Interscience Publishers, New York, N. Y. 1947. Price $6.00.

This book is an extensively revised and expanded portion of the well-known 1928 volume by Kolthoff and Furman. The present volume, *Titration Methods*, is a companion to volume I of the series, *Theoretical Fundamentals*, published in 1942. The book includes the general sections of acid-base reactions and of quantitative precipitation and complex formation reactions. The revised portion of the latter half of the earlier text on oxidation reduction methods is scheduled for publication as volume III in 1948.

The presentation has the same objectives and follows the same pattern as in the earlier volume. The authors give critical, detailed presentations of a somewhat arbitrary selection of methods. In the discussion of procedures the value of the wealth of experience of the authors is apparent. Specific recommendations are sometimes given, but on the whole the authors properly prefer to present different methods or variations together with ample literature references, and to thus allow the analyst to choose the procedure he deems best. In the presentation of methods, emphasis has been on measurement of inorganic or organic constituents of prepared or relatively pure samples. There is little information on the applications of the methods to analysis of biological materials.

The material presented in the original volume has been almost wholly retained; the expansion is due to revision and inclusion of new material. Enlargement of the section on nitrogen determination and presentation of more methods for various organic substances and for fluorine may be of interest to the biochemist. Later procedures are frequently given; the inclusion of a modification of Conway's micro-diffusion method for microdetermination of ammonia is a good example.

The methods are grouped according to the principle involved rather than the substance being determined, the purpose being to emphasize the principle of the determination. The value of this classification is weakened by the difficulty of making unequivocal decisions and by the frequent necessity of classifying a method on basis of only a small part of the procedure. In addition, it is necessary for the user to consult several portions of the volume for the various methods that may be presented for determination of one substance. Cross references in this regard are not always adequate.

The presentations are confined almost entirely to volumetric methods. To many this may be an inconvenient limitation, especially since reference to or mention of other procedures, which may be of equal or greater value than the volumetric method, is not made.

In their grouping of material and presentations the authors use the older established concepts of acids and bases rather than newer definitions based on Brönsted's concepts. Some other omissions of more recent views are apparent. For example, in presenting the formol titration of amino acids, formaldehyde is represented as combining with the amino group to form a Schiff base, in neglect of the considerable evidence against this mechanism. In this same regard the reviewer is entirely unaware of the necessity of the authors to note ". . . an interesting fact commonly ignored in physiological chemistry. The effect of alcohol on amino acids is not directly comparable with that of formaldehyde."

The clarity of presentation has been improved considerably over the earlier volume by use of better sentence structure and by more modern page make-up. Ambiguities and contradictions are rare, as are typographical errors. Some readers may object to the frequent use of relatively small type.

The preceding statements serve only to point out some limitations in an excellent book as evaluated for usefulness to a biochemist. The value and rarity of critical, detailed presentations in methodology, which made the earlier edition of this book a success, assure wide welcome and usage of the present volume.

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The Chemistry of the Carbon Compounds. Volume IV. The Heterocyclic Compounds and Organic Free Radicals. By Victor von Richter. Edited by Richard Anschütz and translated from Volume III and Volume II, part 2, of the 12th German edition by M. F. Darken and A. J. Mee. xv + 498 pp. Elsevier Publishing Co., Inc., New York, N. Y. 1947. Price $12.00.

Richter-Anschütz has long been one of the classical reference texts of organic chemists and it is with regret that one finds that the present translation of the Heterocyclic Compounds and Organic Free Radical sections of the German edition has not been brought up to date. Unfortunately, this volume, like the previously published Volume III of the English edition, has been a victim of the war and the publishers have chosen to print a literal translation of the German edition rather than to delay or discontinue the publication of this series. It is felt that this was an unfortunate decision since as a result the Heterocyclic section covers the literature only through 1931 and the Organic Free Radical section through 1935. Thus the book loses some of its value as a reference text. One valuable feature of the present translation is that wherever possible the references are given to the original journals and not to *Chemisches Zentralblatt*, and names of authors have been added.

The pattern of the book is similar to previous volumes of this series in that chemical reactions, preparations, and properties are given fairly exhaustive treatment, while few theoretical discussions are given. Division is in two major parts, I. *The Heterocyclic Compounds* by F. Reindel (383 pp.) and II. *Organic Free Radicals* by Ludwig Anschütz (69 pp.). The division under part I is based on the number of atoms in the ring with further subdivision according to the number of hetero atoms in the ring. The part on Free Radicals is divided into a short General and a Special Part with the latter being further divided into sections based on the types of free radicals involved, i.e., those of carbon, nitrogen, oxygen, sulfur, chromium, and other elements of the third, fourth, and fifth vertical series of the periodic system.

The book is satisfactorily printed and bound, and although it does not have an author index it has an adequate subject index of 49 pages. The price of $12.00 is somewhat high for the private library when one considers its potential value as an up-to-date reference.

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Technique de la Désinsectisation. By A. L. Lepigre. 270 pp., 31 figures. A. Joyeux, Alger. 1947.

This book is devoted largely to a discussion of the use of fumigants for the control of household and stored product insects. Other phases of fumigation considered are soil fumigation, fruit-tree fumigation and the possibility of using poison gas to combat field infestations of grasshoppers. Information is also included relative to the use of contact and residual sprays in houses, warehouses, and mills and to the use of chemically inert and chemically active dusts for the protection of stored grain.

The subject matter of the various chapters is indicated by the following titles: Chapter 1. Importance of insects to modern man; 2. Principal enemies; 3. Resistance of insects to control methods; 4. Insect protection—control methods; 5. Fumigation of packaged commodities; 6. Insecticidal agents—characteristics—dosages; 7. Physical properties of gases affecting fumigations; 8. Influence of temperature and humidity; 9. Influence of local conditions and stacking of merchandise on the efficacy of fumigants; 10. Materials—Methods—Precautions; 11. List of materials required for mobile fumigator; 12. Detection of HCN and methyl bromide; 13. Gas intoxications—first aid; and 14. Legislation regarding fumigations.

The information contained in the book is of a highly practical nature and is based on the practical experience of the author combined with a careful review of the literature on the subject. The bibliography contains 513 references.

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SUGGESTIONS TO AUTHORS

General. From January 1, 1948, an abstract will be printed at the beginning of each paper instead of a summary at the end, references will be numbered to provide the option of citing by number only, and date of receipt, author's connections, etc., will be shown in footnotes. Except on these points, authors will find the last volume of *Cereal Chemistry* a useful guide to acceptable arrangements and styling of papers. "On Writing Scientific Papers for Cereal Chemistry" (*Trans. Am. Assoc. Cereal Chem.* 6: 1–22. 1948) amplifies the following notes.

Authors should submit two copies of the manuscript, typed double spaced with wide margins on 8½ by 11 inch white paper, and all original drawings or photographs for figures. If possible, one set of photographs of figures should also be submitted. Originals can then be held to prevent damage, and the photographs can be sent to reviewers.

Titles and Footnotes. Titles should be specific, but should be kept short by deleting unnecessary words. The title footnote shows "Manuscript received . . ." and the name and address of the author's institution. Author footnotes, showing position and connections, are desirable although not obligatory.

Abstract. A concise abstract of about 200 words follows title and authors. It should state the principal results and conclusions, and should contain, largely by inference, adequate information on the scope and design of the investigation.

Literature. In general, only recent papers need be listed, and these can often be cited more advantageously throughout the text than in the introduction. Long introductory reviews should be avoided, especially when a recent review in another paper or in a monograph can be cited instead.

References are arranged and numbered in alphabetical order of authors' names and show author, title, journal, volume, first and last pages, and year. The list is given at the end of the paper. Reference numbers must invariably be cited in the text, but authors' names and year may be cited also. Abbreviations for the names of journals follow the list given in *Chemical Abstracts* **40**: I–CCIX. 1946.

Organization. The standard organization involves main sections for abstract, introduction, materials, methods, results, discussion, acknowledgments, and literature cited. Alternately, a group of related studies, each made with different materials or methods, may require a separate section for each study, with subsections for materials and methods, and for results, under each section. Center headings are used for main sections and italicized run-in headings for subsections, and headings should be restricted to these two types only.

Tables. Data should be arranged to facilitate the comparisons readers must make. Tables should be kept small by breaking up large ones if this is feasible. Only about eight columns of tabular matter can be printed across the page. Authors should omit all unessential data such as laboratory numbers, columns of data that show no significant variation, and any data not discussed in the text. A text reference can frequently be substituted for columns containing only a few data. The number of significant figures should be minimized. Box and side headings should be kept short by abbreviating freely; unorthodox abbreviations may be explained in footnotes, but unnecessary footnotes should be avoided. Leader tables without a number, main heading, or ruled lines are often useful for small groups of data.

Tables should be typed on separate pages at the end of the manuscript, and their position should be indicated to the printer by typing "(TABLE I)" in the appropriate place between lines of the text. (Figures are treated in the same way.)

Figures. If possible, all line drawings should be made by a competent draftsman. Traditional layouts should be followed: the horizontal axis should be used for

the independent variable; curves should be drawn heaviest, axes or frame intermediate, and the grid lines lightest; and experimental points should be shown. Labels are preferable to legends. Authors should avoid identification in cut-lines to be printed below the figure, especially if symbols are used that cannot readily be set in type.

All drawings should be made about two to three times eventual reduced size with India ink on white paper, tracing linen, or *blue*-lined graph paper; with any other color, the unsightly mass of small grid lines is reproduced in the cut. Lettering should be done with a guide using India ink; and letters should be $\frac{1}{16}$ to $\frac{1}{8}$th inch high after reduction.

For difficult photographs, a professional should be hired or aid obtained from a good amateur. The subject should be lighted to show details. A bright print with considerable contrast reproduces best, and all prints should be made on glossy paper.

All original figures should be submitted with one set of photographic reproductions for reviewers, and each item should be identified by lightly writing number, author, and title on the back. Cut-lines (legends) should be typed on a separate sheet at the end of the manuscript. "Preparation of Illustrations and Tables" (*Trans. Am. Assoc. Cereal Chem.* **3**: 69–104. 1945) amplifies these notes.

Text. Clarity and conciseness are the prime essentials of a good scientific style. Proper grouping of related information and thoughts within paragraphs, selection of logical sequences for paragraphs and for sentences within paragraphs, and a skillful use of headings and topic sentences are the greatest aids to clarity. Clear phrasing is simplified by writing short sentences, using direct statements and active verbs, and preferring the concrete to the abstract, the specific to the general, and the definite to the vague. Trite circumlocutions and useless modifiers are the main causes of verbosity; they should be removed by repeated editing of drafts.

Editorial Style. A.A.C.C. publications are edited in accordance with *A Manual of Style*, University of Chicago Press, and *Webster's Dictionary*. A few points which authors often treat wrongly are listed below:

Use names, not formulas, for text references to chemical compounds. Use plural verbs with quantities (6.9 g. were). Figures are used before unit abbreviations (3 ml.), and % rather than "per cent" is used following figures. All units are abbreviated and followed by periods, except units of time, which are spelled out. Repeat the degree sign (5°–10° C.). Place 0 before the decimal point for correlation coefficients ($r = 0.95$). Use * to mark statistics that exceed the 5% level and ** for those that exceed the 1% level; footnotes explaining this convention are no longer required. Type fractions on one line if possible, e.g., $A/(B + C)$. Use lower case for farinograph, mixogram, etc., unless used with a proper name, i.e., Brabender Farinograph. When in doubt about a point that occurs frequently, consult the Style Manual or the Dictionary.

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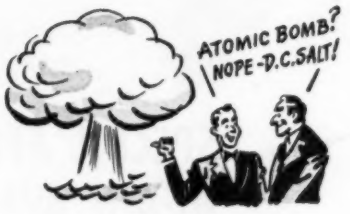

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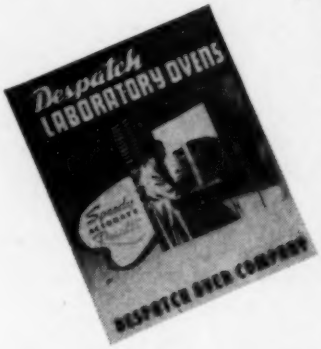

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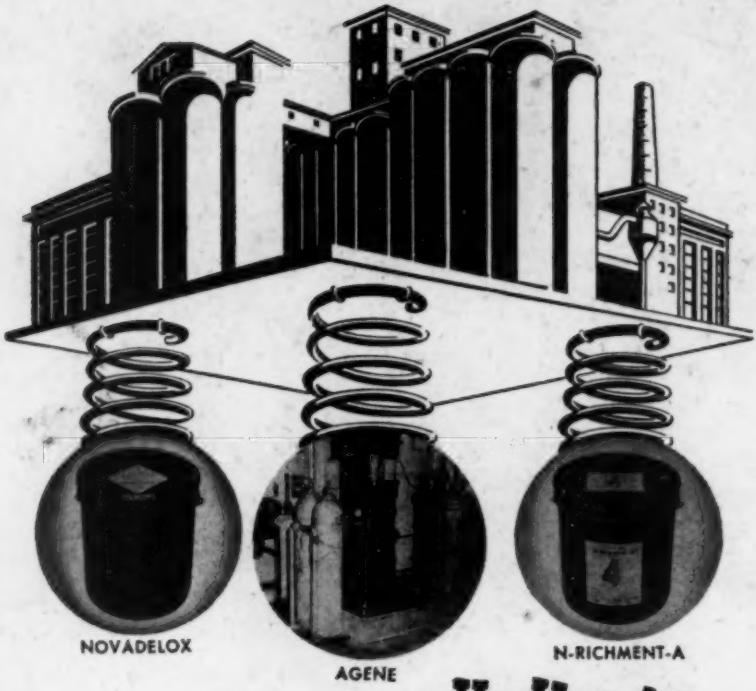

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